Test Methods
for the Examination of
Composting
and
Compost

JOINT PROJECT OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE UNITED STATES COMPOSTING COUNCIL

Prepared for:
THE US COMPOSTING COUNCIL RESEARCH AND EDUCATION FOUNDATION, AND
THE UNITED STATES DEPARTMENT OF AGRICULTURE

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DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org/addenda.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

MANUAL DEVELOPMENT

This manual of test methods has benefited from the expertise and input of numerous individuals. In addition, several groups cooperated and many contributors provided valuable suggestions for improvement. Throughout its development, the focus always remained on producing a technically sound manual of test methods and protocols. The development stages of the TMECC project are described below.

Stage 1. Project Concept Development

The US Composting Council's Standards and Practices Committee and Markets Development Committee developed a list of key process variables to measure and manage, and attributes to report for the following: composting feedstock; the composting process; finished compost, product safety and regulatory compliance, and marketing claims.

Stage 2. Minnesota Compost Utilization Project (MN-CUP)

The Standards and Practices Committee worked cooperatively with the Minnesota Office of Environmental Assistance, the University of Minnesota's Department of Soil, Water, and Climate Research Analytical Laboratory, and Malcolm Pirnie for two years to survey eight municipal solid waste composting facilities in Minnesota. This was an observational study designed to document feedstock, in-process and finished compost sampling and preservation protocols, laboratory preparation steps and analytical methodologies. The documented sampling and analytical methods are presented along with others in this manual in the form and style of ASTM methods.

The MN-CUP study was divided into three phases:

Phase I—Sample Variability. For the first two months, three separate compost samples were collected at each of three locations to document variability within one batch at one site. One of the three samples from each location was subdivided into ten subsamples during laboratory sample preparation to evaluate within-sample variability.

NOTE—A Reference Sample (in-house) of municipal solid waste compost was created with excess material from one of the original three locations. This material was first air-dried at 36°C, sieved through a 4-mm sieve and milled with a Stein mill (carbide-tipped blade). The milled material was oven-dried at 70°C to minimize enzymatic degradation, mixed in a tumble blender for 2 d, split with a sample splitter and stored in 2 L polyethylene bottles at room temperature (~28°C).

Phase II—Temporal Variability. Sampling continued on a monthly basis for one year at the previously mentioned sites. Five additional facilities were added for the duration of the first year's monthly sampling phase.

Phase III—Temporal Variability. Sampling frequency was decreased to a quarterly basis for seven facilities during the second year of sampling. One facility was lost to fire.

Test Parameters from MN-CUP

During the MN-CUP project, approximately 40 parameters were considered. The methods were modified and adapted from existing ASTM, ASA-SSSA, SW-846 and AOAC methods developed for other materials. Test parameters considered:

I. *Chemical Analyses*—using US EPA 3051 digest modified for compost's high organic matter and ICP-AES determinations for metals and salts; cold vapor for Hg; wet combustion determination for N (total Kjeldahl nitrogen, micro-digest technique); colorimetric NO_3 and NH_4; and cation exchange capacity (modified ammonium displacement technique on milled material).

II. *Physical Analyses*—for total solids and moisture (wet basis); ash (volatile solids); man-made inerts (plastics, metal, glass); bulk density; water-holding capacity; and air-capacity.

III. *Biological Analyses*—for stability (oxygen uptake); growth and germination (a direct seeding technique); and organic carbon using dry combustion.

IV. *Pathogens Analysis*—included fecal coliforms (determined at private laboratories outside of the University of MN system).

V. *Organics Analyses*—included volatile fatty acids and polychlorinated biphenyls (PCB's determined at private laboratories outside of the University of MN system).

Stage 3. Draft of Sampling and Analysis Protocols

A scientifically based catalog and laboratory manual of methods was drafted for use with feedstock and compost analysis to initiate the standardization process for regulatory and market requirements, and management of the composting process.

This work included formatting, enhancement and critical review of methods devised and modified at the University of Minnesota's Department of Soil, Water and Climate Research Analytical Laboratory, St. Paul,

by Robert Munter's group and private laboratories for the MN-CUP project. Other methods developed during parallel compost projects were added to complement the MN-CUP work, funded by the Composting Council Research and Education Foundation and The Procter & Gamble Company are included in the manual.

Goal—To provide a science-based manual of rigorous test methods specifically appropriate to feedstocks and finished compost, as distinct from soil, manure, and fertilizers, and applicable to regulatory and market requirements, and to augment these methods with a suite of *quick tests* for managing the composting process.

Stage 4. Introduction and Peer Review—December 1997

Goal—Introduce TMECC to the composting community and familiarize users with its intended purpose and content; solicit feedback to refine and expand manual content. A review draft of TMECC was provided to approximately 160 laboratories, compost production facilities, and academic institutions. Collaborating groups and individuals were requested to perform the following:

4.1 analyses of composts by methods provided in TMECC,

4.2 document commentary and critiques of existing methods, and

4.3 solicit for and add missing test methods.

Product—Revision of the First Draft of *Test Methods for the Examination of Composting and Compost*.

Stage 5. Collaborative Evaluation, On-Going

Goal—Develop consensus for test definitions:

5.1 provide replicated samples of composts from varying feedstock types and combinations; include a minimum of three laboratories per test to establish method precision with resulting data to be used in precision tables to identify and document sources of bias,

5.2 synthesize user feedback to identify and document consensus among participating laboratories for acceptance of at least one test method for each test parameter where appropriate,

5.3 remove antiquated methods, and

5.4 solicit for and add missing test methods.

Products—Reference Editions of Test Methods for the Examination of Composting and Compost.

Stage 6. USDA Greenhouse and Field Testing

Goal—Identify and document correlation among different test method values and calibrate tests to obtain interpretive information about using the compost.

Product—Test interpretation guidelines for compost application management.

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ABBREVIATIONS

Oac	-acetate
ACS	America Chemical Society
ASTM	American Society for Testing Materials
ASA	American Society of Agronomy
Å	Ångström units (10^{-8} cm, or 0.1 nm)
AshW	ash weight determined at 550°C
AOAC	Association of Official Analytical Chemists
Atm	Atmosphere(s), 1 atm = 101,325 Pa
AA	atomic absorption
cm	centimeter(s)
cmol	centi mol(s), cmol kg^{-1} ≡ meq 100 g^{-1}
CCFREF	Composting Council Research and Education Foundation
C	coulomb
d	day(s), time
Δ	delta, change, or difference
°C	degrees Celsius
°F	degrees Fahrenheit °C = 5 ÷ 9 × (°F – 32)
dw	dry weight basis, equal to TS basis
÷	divided by, division symbol
dS	deci-Seiman, equal to dMhos
EC	Enzyme Commission
=	equal to
≡	equivalent to
EtOH	ethanol
Fig	figure, illustration, chart, drawing, diagram
ft	foot (feet) (30.480061 cm)
e.g.	for example
gal	gallon(s), US liquid (3.7853 L)
GC	gas chromatography
g	gram(s)
g	gravitational force, cm sec·sec^{-1}, ft sec·sec^{-1}
>	greater than, more than, exceeds
h	hour(s), time
in.	inch(es) (2.54 cm)
ICP-AES/M	inductively coupled plasma - atomic emission spectroscopy/mass spectroscopy
i.d.	inner diameter (dimension)
ISO	International Organization for Standardization
kg	kilogram(s)
<	less than, under, below
L	liter(s), liquid
mhos	unit of conductance (Sieman's unit, Ω^{-1})
MS	mass spectrometry
MΩ	megohm(s)
m	meter(s)
µg	microgram(s)
µL	microliter(s)
µm	micrometer(s)
meq	milli equilivent(s)
mL	milliliter(s)
mm	millimeter(s)
mMhos	milliMhos, equal to mS
ms	millisecond(s)
min	minute(s), time
M	mega
MSW	mixed municipal solid waste
MMW	mixed municipal waste
M	molar
MW	molecular weight
MSW	municipal solid waste
ng	nanogram(s)
nm	nanometer(s)
USCC	US Composting Council, The
NIH	National Institute of Health, US
N	normal concentration
No.	number, #
OM	organic matter
Ω	ohm, unit of resistance (1 $Mhos^{-1}$)
oz	ounce(s) US fluid (0.02957 L)
o.d.	outer diameter (dimension)
dw	oven-dry weight basis determined at 70±5°C
ODW	oven-dry weight basis determined at 70±5°C
Pa	pascal(s)
ppb	parts per billion (1 x 10^9), e.g., µg kg^{-1}
ppm	parts per million (1 x 10^6), e.g., mg kg^{-1}
%	percent (parts per 100); percentage
pt	pint(s), US liquid (0.4732 L)
TD	pipette volume to deliver
lb	pound(s) (453.6 g)
psi	pounds per square inch (0.06805 atm)
PRS	process to reduce sharps
qt	quart(s), US liquid (0.9463 L)
s	second(s), time
S	Seiman, equal to Mhos
rpm	revolutions per minute
SSSA	Soil Science Society of America
STP	standard temperature (25°C) and pressure (101,325 Pa)
t	time
×	times, multiplication symbol
USDA	United States Department of Agriculture
US EPA	United States Environmental Protection Agency
W	watts
yd	yard(s) (0.9144 m)
SM	Standard Methods for the Examination of Water and Wastewaters

MANUAL FORMAT

1. Test Method Categories

1.1 The test methods presented in TMECC are separated into seven [7] chapters categorized by sample collection and preservation (02.00), physical attribute tests (03.00), chemical analysis (04.00), and organic and biological determinations (05.00), with references to pertinent synthetic organic chemicals determination methods (06.00) and pathogen testing procedures (07.00). Each test method is designed for analyzing compost materials at one or more of the six [6] composting process steps described in chapter 01.00, and to document compost safety standards or market attributes.

2. Test Method Coding System

2.1 *Alpha-Numeric Test Method Codes*—Each test method code contains two integers and one hyphenated letter. The first integer identifies the chapter and the second integer represents the test parameter, while the hyphenated letter represents one of various possible test methods that may be used for the measurement, or determination of a test parameter.

EXAMPLE 1—the code "05.08-D" represents test method "D" of test parameter eight [8] in chapter five [05].

2.2 *Referenced Methods*—Test methods of interest may not be included in TMECC because:

2.2.1 the method is proprietary;

2.2.2 methods are well documented in other manuals; or

2.2.3 the method has not yet been adequately optimized for use with composting materials.

2.3 *Page Numbering*—Page numbers are located on the outside lower corner of each page. The page number is preceded by the hyphenated chapter number and section number.

EXAMPLE—"02.01-8" represents page eight of section one [1] in chapter two [2].

2.4 *Figures and Tables*—The alpha-numeric code for test methods is expanded to include an additional number following the hyphenated letter. Both figures and tables are numbered from one for each test method. The first number indicates the chapter, the second number indicates the test parameter, and the letter corresponds with the test method, while the last number indicates the figure or table within a method.

EXAMPLE—Fig 04.02-A1 Conceptual example of a standard addition plot.

EXAMPLE—Table 04.04-A1 General interpretation guidelines for greenhouse growth media analyzed by the Saturated Media Extract method (dS m^{-1}).

3. Test Method Page Format

3.1 A test method applications guide is provided as the header for each test method to indicate which methods are appropriate for each of the six composting process steps. Test methods are represented by alpha-numeric code by column under each process step.

3.2 Each test parameter is presented in three parts:

3.2.1 parameter introduction and background;

3.2.2 procedural outlines where more than one procedure may be presented for a parameter; and

3.2.3 method summaries.

3.3 An abbreviated test method application guide for each test method is provided on the first page of each method.

4. Method Guide Format

4.1 The application guide headings provide the following test method information (Fig 00.01-1).

4.1.1 *Test Parameter*—product attribute, such as pH, total solids, etc.

4.1.2 *Test Method*—analytical procedure or quick test for measuring the parameter.

4.1.3 *Reporting Units*—reporting units and moisture basis, such as mg kg^{-1} dw, g g^{-1} % wet basis, g cm^{-3} dw, etc. Refer to the list of abbreviations presented in this preface for a description of each abbreviation used in this manual.

4.1.4 *Test Method Applications*—Test method codes are inserted where analysis is appropriate for the indicated process management steps, or safety and market attributes (detailed in chapter one).

Test Method: *Parameter* (*see 4.1.1*). *Test method* (*see 4.1.2*)						**Units:** *(see 4.1.3)*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
(see 4.1.4)								

Fig 00.01-1 Test method applications guide.

SAMPLE FATE CHART

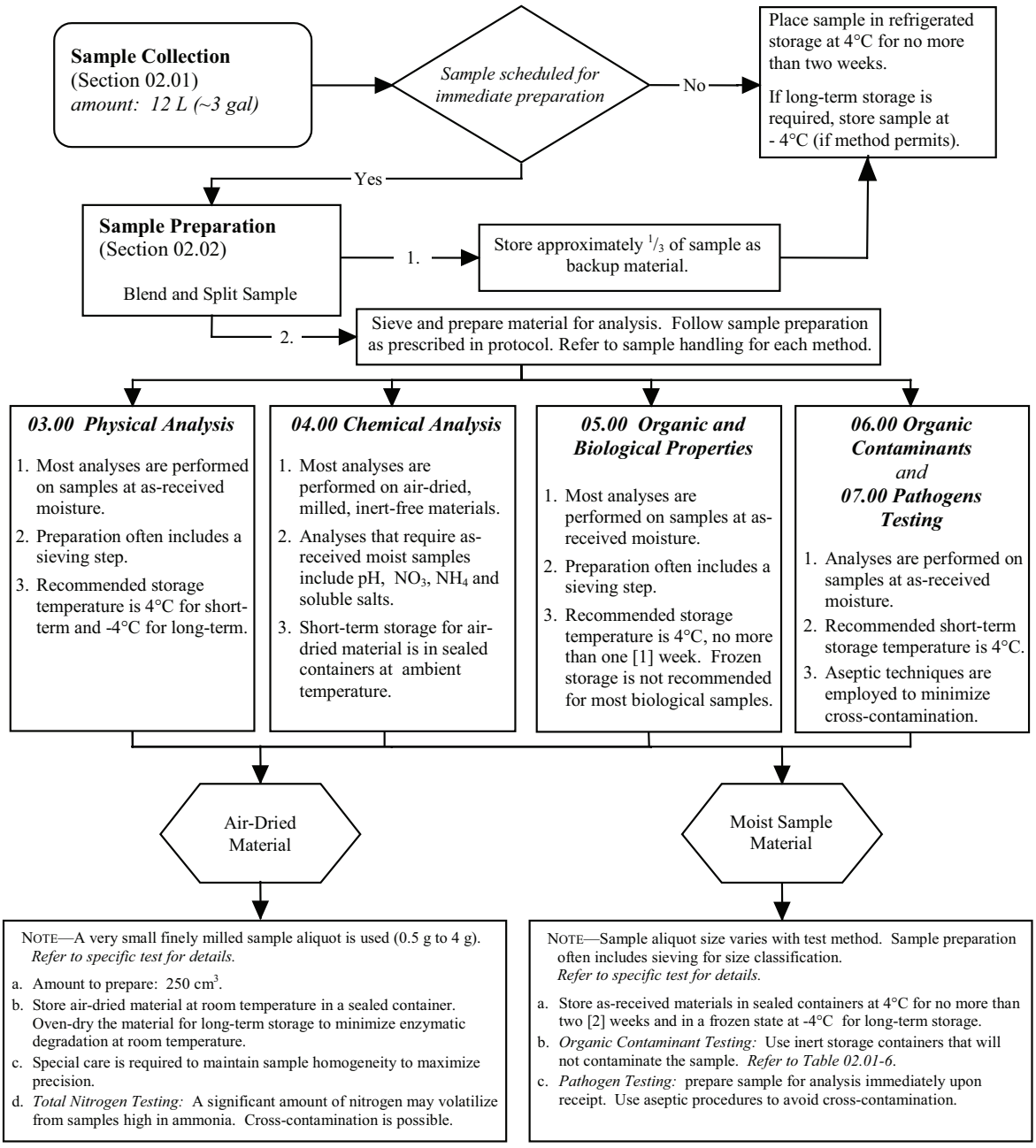

Fig 00.01-2 Fate chart of sample flow from collection through laboratory preparation and analysis.

TMECC

Test Methods for the Examination of Composting and Compost

Purpose

Test Methods for the Examination of Composting and Compost (TMECC) provides detailed protocols for the composting industry to verify the physical, chemical, and biological condition of composting feedstocks, material in process and compost products at the point of sale. Material testing is needed to verify compost product safety and market claims. TMECC provides protocols to sample, monitor, and analyze materials at all stages of the composting process, (e.g., prior to, during and after composting), to help maintain process control, verify product attributes, assure worker safety, and to avoid degradation of the environment in and around the composting facility.

Standardized methods to characterize compost are needed by compost producers, state regulatory and permitting agencies, compost product marketing specialists, state and commercial testing laboratories, and agriculturalists, horticulturalists, landscapers, and other consumer sectors. Use of standard methods and protocols for sampling, laboratory analysis, reporting, and interpretation of test results will promote production and marketing of quality composts that meet a core set of analytical standards.

TMECC is approved for publication through the USGPO as part of USDA's Conservation Resources Technical Bulletin Series.

Overview of TMECC Development

Summary

TMECC was jointly published by the US Department of Agriculture (USDA) and the Composting Council Research and Education Foundation (CCREF). The TMECC Project was initiated by The Procter and Gamble Company in mid 1995 under the direction of Phil B. Leege, and adopted by the Composting Council Research and Education Foundation in late 1995 under the leadership of Dr. Charles Cannon, former Executive Vice President of the Composting Council. Refer to Fig 1 through Fig 3 for diagrams that illustrate key participants and their responsibilities during the TMECC development and peer-review process.

TMECC evolution and the TMECC Project is categorized by six developmental stages: i) draft of methods; ii) compilation of methods; iii) content peer-review; iv) round-robin testing; v) addition of interpretation and end-use guidelines; and vi) maintenance and addenda distribution.

The initial draft of methods was completed in December 1995 and the enhanced compilation of methods was completed in December 1997. TMECC content peer-review was initiated in March

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[1] The 2002 Compost Analysis Proficiency (CAP) Testing Program is managed by Robert O. Miller. CAP was established, in part, as a vehicle to measure performance, and to examine the credibility of TMECC. Visit http://tmecc.org/cap/ for detailed information.

1998 and formally completed in August 2001. Proficiency testing is underway and was implemented as follows: a preliminary round-robin using triplicate samples from 15 composting facilities and three laboratories was carried out in 2000 through collaboration with the USCC's Seal of Testing Assurance[2] (STA) program and USDA-ARS-BARC-SASL (Fig 4); in 2001 and 2002, round robin-testing was expanded to include 23 laboratories through collaboration between Compost Analysis Proficiency (CAP), STA and the TMECC Project (Fig 5).

Stage six of TMECC development will incorporate the proficiency testing program as part of a new and expanded peer-review process; data generated through proficiency testing will serve as a feedback mechanism used to update and maintain TMECC. CCREF is seeking formal relationships with professional organizations to fulfill the need for critical technical oversight of the peer-review process. Refer to Fig 6 and Fig 7 for diagrams that illustrate proposed feedback and TMECC maintenance mechanisms.

Stages I and II ▫ Compilation

The first stage of the TMECC Project which spanned approximately three years was principally funded by The Procter and Gamble Company and the MN Office of Environmental Assistance. This effort included bench-level evaluation of existing test methods at the U of MN Research Analytical Laboratory. Tests were performed on samples of source separated and mixed municipal solid waste composts. This effort involved modification of existing test methods developed for other materials, (e.g., soils, water, biosolids, etc.). A preliminary draft of TMECC was compiled using test methods employed at the U of MN and later expanded to include other generally accepted methods plus a small number of new techniques for analyzing compost parameters which had no generally accepted or readily available method. The product of the first stage of the TMECC Project was a 1,000-page peer-review draft entitled "The First Edition of TMECC".

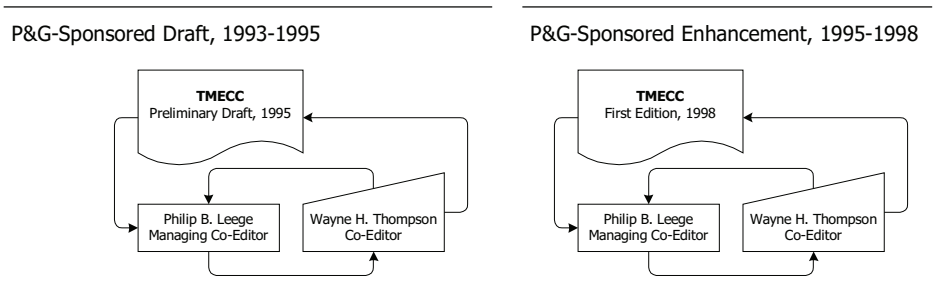

Fig 1. Stages I and II of the TMECC Project. The preliminary draft was compiled and later expanded to include additional methods. The final product of this effort was the peer-review draft, "First Edition of TMECC".

Stage III ▫ Peer-Review of the First Edition

The third stage of the TMECC Project was a coordinated peer-review of TMECC, the 1,000-page volume developed during stages one and two of the project (Fig 2). The peer-review process spanned a period of four years and was principally funded by the USDA.

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[2] The 2002 Seal of Testing Assurance (STA) is the USCC composting product data disclosure and end-use guideline program for compost producers and distributors. The program targets compost producers and is intended to promote the use of established compost sample collection and testing procedures, and to indicate appropriate end-use guidelines for their tested compost products. Visit http://tmecc.org/sta/ for detailed information.

TMECC sections were grouped into nine academic categories. A group leader was recruited to manage each review category. Each group leader recruited his/her team of peer-reviewers. The first edition of TMECC was then distributed to more than 175 reviewers selected for category-specific feedback. Individual reviewer input was compiled by each group leader and incorporated into TMECC by the Editor-in-Chief. The revised sections were then approved by the co-editors and re-submitted to the group leaders for their acceptance of the final draft of their assigned sections.

The peer-review process evolved into a rigorous re-write of TMECC. The latest USGPO working draft incorporates comprehensive critiques from over 175 compost analytical experts from around the world. This completely revised work was submitted to the USDA in December 2000 as a project deliverable; USDA extended the review process before granting its approval and acceptance of TMECC as a compost sampling and laboratory manual.

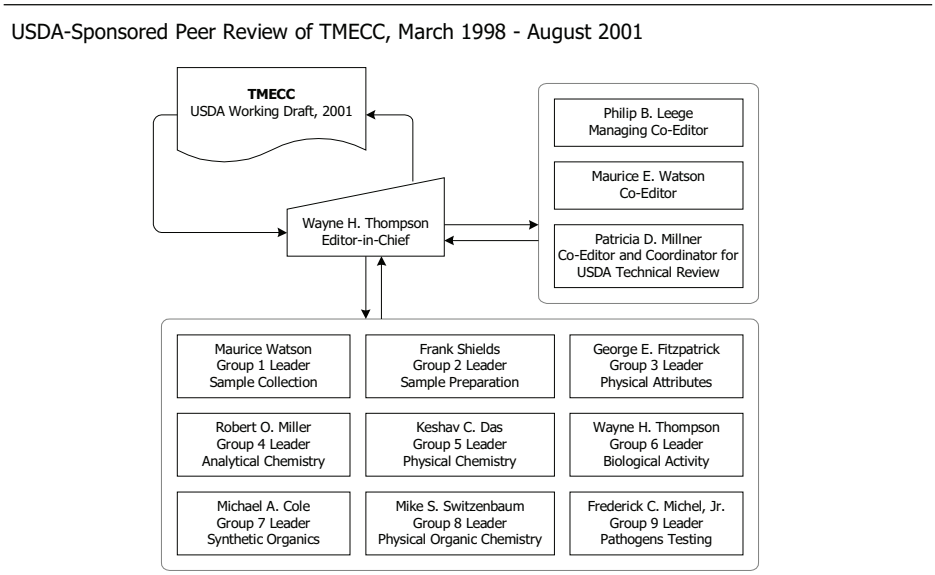


Fig 2. Stage III of the TMECC Project. The peer-review process was initiated in March 1998. USDA Technical Review was initiated in December 2000 and completed in August 2001.

Dr. Patricia Millner supervised the USDA internal review and worked directly with Editor-in-Chief of TMECC to implement all modifications required by USDA. Dr. Millner presented a prospectus to publish TMECC as a USDA Conservation Resources Technical Bulletin to the USGPO in October 2000; the prospectus to publish was approved in March 2001. The prospect of releasing TMECC as a USGPO document prompted USDA to intensify the review process which extended the Stage III completion date by an additional nine months. The USDA internal technical review was formally completed in August 2001 and the working draft was submitted to the USGPO editorial staff in August 2001. Refer to Fig 3, and Appendix I for the list of methods provided in TMECC.

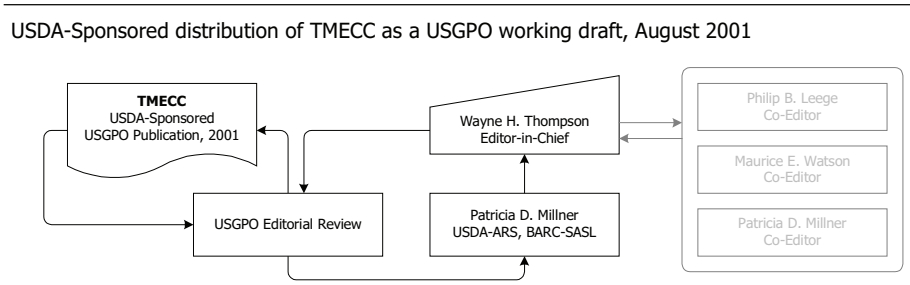


Fig 3. USGPO Review Process – The last step in Stage III of the TMECC Project.

Stage IV ▫ Laboratory Proficiency Testing

Laboratory proficiency testing is a QA/QC tool used by laboratory personnel to verify their analytical performance relative to other laboratories that use common methods and analytical protocols for specified parameters. The Compost Analysis Proficiency Testing program (CAP) was initiated through collaboration with managers of the North American Proficiency Testing Program (NAPT) to provide the compost laboratory analysis industry with an inter-laboratory QA/QC program, to develop reference materials, and to provide comparative data needed to measure performance and precision of TMECC analytical methods.

Laboratory proficiency testing is the fourth stage of the TMECC Project and is considered on-going. The USDA-Sponsored Seal of Testing Assurance Pilot (STA-2000) was implemented to serve as a preliminary round-robin designed to reveal operational oversights in common test methods. This was implemented prior to the formation of CAP and prior to the submission TMECC as a project deliverable to USDA. Refer to Fig 4 for the list of participants. Preliminary examination of the USDA-sponsored round robin results provided crucial information which allowed the TMECC editors to clarify important steps in various laboratory protocols with a resulting improvement in across-lab precision.

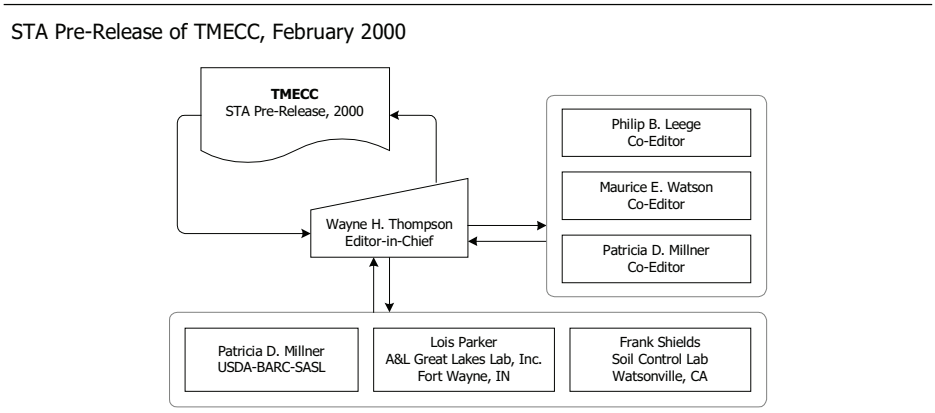


Fig 4. Stage IV of the TMECC Project. Preliminary round-robin testing was carried out by three laboratories on triplicate compost samples from fifteen facilities.

Round-robin testing of TMECC was expanded in 2001 and 2002 to include participating CAP laboratories (Fig 5). Preliminary results from the first 2001 sample exchange of the 2001 CAP program were not conclusive; results from later exchanges are needed to construct a more meaningful data set; and additional steps were implemented to incorporate more descriptive and detailed performance data reporting and comments from participating laboratory personnel. Participation in CAP is expected to increase after TMECC is released as a USGPO document.

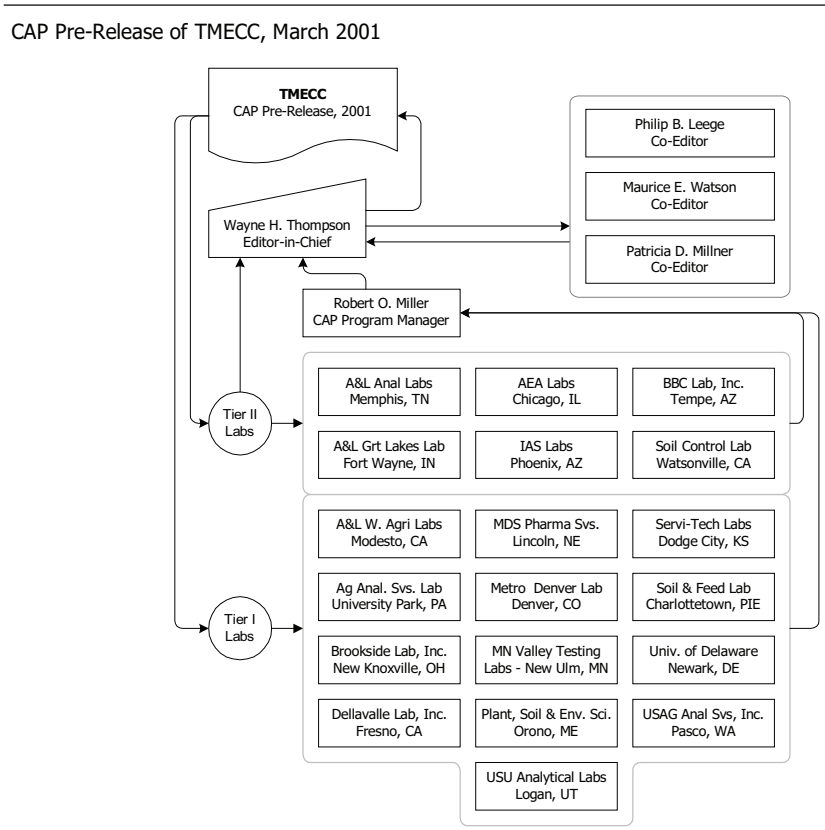


Fig 5. Stage IV of the TMECC Project. Round-robin testing through the CAP testing program.

Stage V ▫ Interpretation and End-Use Guidelines

The fifth stage of the TMECC Project is considered on-going. Interpretation guidelines were incorporated during Group 6 peer-review for two biological activity sections, the respirometry (05.08) and indicator ratios (05.02) sections. Much more effort must be expended and information compiled before this aspect of TMECC can be considered comprehensive.

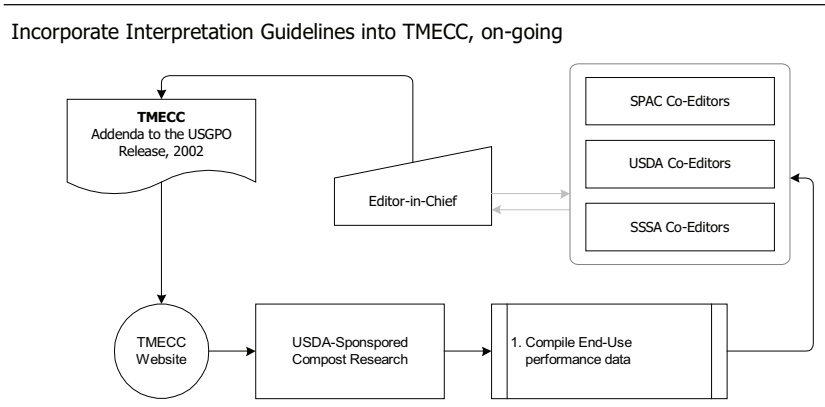


Fig 6. Stage V of the TMECC Project. Incorporation of interpretation guidelines.

Stage VI ▫ Maintenance and Updates, PROPOSED for 2002 and Later

The sixth stage of the TMECC Project will begin after TMECC is formally released through the USGPO as a USDA document. The Standards and Practices Committee of the US Composting Council is in the process of forming oversight subcommittees. Subcommittees will consist of compost analytical specialists who will provide technical oversight for the maintenance and enhancement of TMECC. The effort to form category-specific review subcommittees requires solicitation of technical assistance, paid and/or voluntary, from current participants of the TMECC review process and other qualified individuals associated with participating organizations, (e.g., Soil Science Society of America [SSSA], Soil and Plant Analysis Council [SPAC], etc.). The primary source of performance data and technical feedback will be collected through the CAP programs; secondary sources may include direct comment via feedback mechanisms available on-line at the TMECC web site, (e.g., discussion list, on-line manuscript submissions, etc.). Pending the availability of project funds, analysis of CAP results will be expanded to include site visits and a review process assembled to evaluate laboratory interpretation of TMECC methods. Finally, mechanisms to create TMECC addenda will be established to provide addenda to the compost analytical community (Fig 6 and Fig 7).

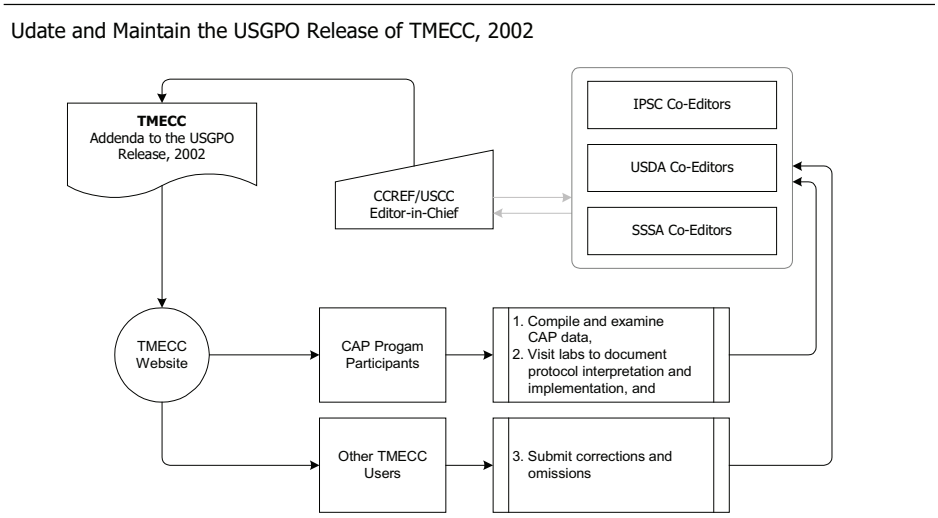


Fig 7. Stage VI of the TMECC Project (PROPOSED). Maintenance and updates of TMECC through feedback and review mechanisms.

Appendix I: TMECC Contents

01.01 TMECC MANUAL CONTENT

01.01-A PURPOSE

1. Compost Quality Verification

1.1 *Test Methods for the Examination of Composting and Compost*—TMECC provides detailed protocols for the composting industry to verify the physical, chemical, and biological condition of composting feedstocks, material in process and compost products at the point of sale. Material testing is needed to verify product safety and market claims. TMECC provides protocols to sample, monitor, and analyze materials at all stages of the composting process, i.e., prior to, during and after composting to help maintain process control, verify product attributes, assure worker safety, and to avoid degradation of the environment in and around the composting facility.

01.01-B FOREWORD

2. Manual Development

2.1 *Test Methods for the Examination of Composting and Compost*—TMECC is a laboratory manual modeled after *American Society for Testing and Materials* (*ASTM)*. TMECC provides benchmark methods for compost analysis to enable comparison of analytical results. Each parameter is presented in its own section and generally includes more than one protocol or test method. The manual contains more parameters than might be of concern or interest for a particular situation.

2.2 *The Standards and Practices, and Market Development Committees of the US Composting Council* developed a list of pertinent compost product attributes. Some compost parameters are regulated for the protection of public health, safety, and the environment, while others are product performance attributes that are important for managing specific applications. Other test parameters and their methods are of academic interest for research applications.

2.2.1 Detailed instruction is presented for sample collection, preparation, analysis and reporting to address all phases of composting, feedstock evaluation, the composting process, and final compost product characterization.

2.2.2 Sections are grouped into chapters that cover sampling and sample preparation (02); physical properties (03); inorganic chemical properties (04); organic and biological properties (05); organic contaminants (06); and pathogens (07).

2.2.3 Each section includes a brief description of the parameter's function in the composting process, for safety of the product, or in product performance.

01.01-C REFERENCED METHODS

3. List of Sources

3.1 Testing procedures included in TMECC were adapted for compost analysis and are based upon, or developed from the following reference sources:

Analytic procedures used in US EPA Report SW-846, Test Methods for Evaluating Solid Waste, 3rd Edition, November 1990, as revised.

Association of Official Analytical Chemists (AOAC) Official Methods of Analysis, 1990, 15th edition.

Methods of Soil Analysis, Parts I, II and III. Soil Science Society of America. 1996

North Central Regional (NCR) Publication No. 221 (Revised). Recommended Chemical Soil Test Procedures for the North Central Region Bulletin No. 499 (Revised) October 1988 "Recommended Test Procedures for Greenhouse Growth Media".

Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1992.

The American Society for Testing and Materials (ASTM) Standard Test Methods, 1988.

01.02 THE COMPOSTING PROCESS

01.02-A KEY PROCESS VARIABLES

1. Management of Key Process Variables

1.1 Process control parameters include initial carbon to nitrogen and carbon to phosphorus ratios, pile structure and porosity, pile oxygen percent, pile moisture percent, and pile temperature. Process management includes aggregate size distribution (particle size), additives and amendments, biological activation and microbial diversity, turning and mixing, aeration, pH and pathogen and weed seed reduction. Odor production must be minimized and in many cases treated before discharge. Odor management is discussed in two parts, odor control and odor treatment. The management of process control operating parameters is presented in the *Compost Facility Operating Guide,* US Composting Council, 1994 and in *Municipal-Scale Composting: A decision Makers Guide to Technology Selection - Composting for Municipalities; Planning and Design Considerations*, Natural Resource, Agriculture, and Engineering Service, Cornell University, 1998 (Bulletin NRAES-94).

1.2 *Key Process Variables* define conditions in the pile for composting. The *Key Process Variables* must be managed during the composting process to ensure consistent marketable product in the shortest time see Table 01.02-A1 Management of Key Process Variables.

1.2.1 *Pile Porosity* is a measure of the space between adjacent particles needed for circulation of air and a film of moisture that surrounds particles.

1.2.2 *Feedstock Nutrient Balance* is the carbon to nitrogen ratio [C:N] and initial carbon to phosphorus ratio [C:P] needed for vigorous microbial activity and complete degradation/stabilization.

1.2.3 *Pile Oxygen Percent* indicates the exchange of carbon dioxide and other gasses generated through microbial metabolic activity. An adequate supply of oxygen promotes aerobic activity that minimizes odor generation associated with anaerobic conditions.

1.2.4 *Pile Moisture Percent* is measured to ensure that sufficient moisture is available within and around each particle such that pores are not completely filled with water (leading to anaerobic conditions) but sufficient to create a moisture film around each particle that supports microbial growth and enzymatic activity. Pile moisture above 35% will aid in dust control.

1.2.5 *Pile Temperature* is a measure of heat generation by catabolic activity of thermophilic bacteria. A sustained pile temperature above 55°C kills pathogens and most weed seeds. Eventually, after significant degradation of the readily available organic matter, pile temperatures decrease and a period (curing stage) ensues during which mesophilic microbes (actinomycetes, bacteria, and fungi) begin the slower rate decomposition of the less readily available energy sources (hemicellulose, cellulose, lignocellulose and lignin) .

1.2.6 *Retention Time* under various temperature regimes ensures full stabilization of feedstocks, and full degradation of organic phytotoxins that form during the early stages of composting and during the initial stages of curing.

Table 01.02-A1 Management of Key Process Variables.

KEY PROCESS VARIABLES	FEED-STOCK	PROCESSING STEPS						
	Feedstock Collected and Delivered	***Step 1:*** Feedstock Recovery	***Step 2:*** Feedstock Preparation	***Step 3:*** Composting	***Step 4:*** Odor Treatment	***Step 5:*** Compost Curing	***Step 6:*** Compost Screening and Refining	***Step 7:*** Compost Storing and Packaging
Pile Porosity	✓	✓	✓	✓	✓	✓	✓	✓
Feedstock Nutrient Balance	✓	✓	✓					
Pile Oxygen Percent			✓	✓	✓	✓		✓
Pile Moisture Percent			✓	✓	✓	✓	✓	✓
Pile Temperature				✓		✓		
Retention Time			✓	✓	✓	✓		✓

2. Product Attribute Check-List

2.1 A check list of product attributes, feedstock choices, and process steps where compost producers can intervene to manage and control product attributes is presented in Table 01.02-A2 Management of Product Attribute Development.

2.2 An appropriate sampling and testing plan must be designed specifically for each process step and each finished product. Refer to *TMECC 02.01 Sample Collection and Preparation* for guidance.

Table 01.02-A2 Management of Product Attribute Development.

PRODUCT ATTRIBUTES	MANAGEMENT OF PRODUCT ATTRIBUTE DEVELOPMENT							
	Feed-Stock	**Processing Steps**						
	Feedstock Collected and Delivered	***Step 1:*** Feedstock Recovery	***Step 2:*** Feedstock Preparation	***Step 3:*** Composting	***Step 4:*** Odor Treatment	***Step 5:*** Compost Curing	***Step 6:*** Compost Screening and Refining	***Step 7:*** Compost Storing and Packaging
SAFETY REQUIREMENTS								
Regulated elements	✓	✓	✓	✓		✓	✓	
Pathogens			✓	✓		✓		✓
MARKET ATTRIBUTES								
Man-made inerts	✓	✓					✓	
Growth screening	✓	✓	✓	✓	✓	✓		✓
Biological stability	✓	✓	✓	✓	✓	✓		✓
Organic matter content	✓	✓	✓	✓	✓	✓		
pH	✓	✓	✓	✓	✓	✓		✓
Soluble salt content	✓	✓						
Water-holding capacity	✓	✓	✓	✓	✓	✓	✓	
Bulk density			✓	✓	✓	✓	✓	✓
Sieve size and Porosity			✓	✓	✓	✓	✓	
Moisture content			✓	✓		✓	✓	✓
Plant nutrient content	✓	✓	✓	✓	✓	✓		✓

3. Process Steps and Test Method Selection

3.1 The composting process can be generally characterized by Fig 01.02-A1 Composting Process Model.

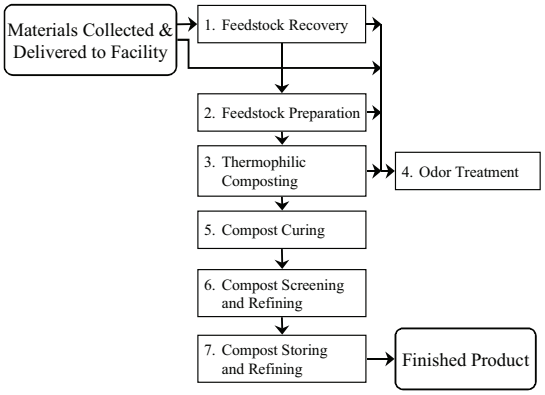


Fig 01.02-A1 Composting Process Model.

3.2 Processing steps 1, 3, 5, 6, and 7 increase the value of finished product, and require some investment in equipment, time and labor. Parts of these steps may include specific criteria to comply with operating permit requirements, (e.g., pathogen treatment during step 3 composting, and perhaps during step 5 compost curing). The optional portions that can increase market value should be undertaken only if the value added can be covered by sales returns. The Composting Products Model (Fig 01.02-A2) shows sources for products that may match customer and user needs without adding unwanted and unnecessary value that will not be compensated with product sales revenue.

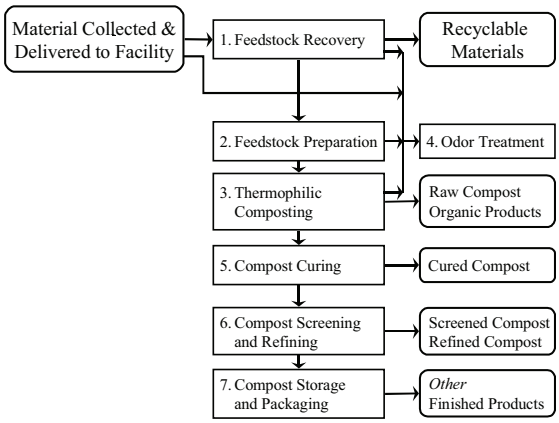


Fig 01.02-A2. Composting Products Model

3.3 Sampling and testing of feedstock and material undergoing biological degradation is required to provide the necessary data for process control.

3.4 Test methods were developed to manage the broad range of data needed by processors and marketers. Each test method is introduced with a header; refer to Table 01.02-A3 Test Method Header. The header shows the Test Method name and Units of measurement and a checklist of Test Method Applications either to the management of process unit operations steps one through seven, and verification of

Product Attributes for compliance with safety standards and market specifications.

Table 01.02-A3 Test Method Header.

Test Method: Parameter. Test Name							**Units:**	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*

4. Process Management Discussion

4.1 *Step 1: Feedstock Recovery*—Recovery of feedstock is the separation of compostable material from non-compostable material, hazardous waste, and other contaminants that can impact compliance with compost quality requirements. Recovery can occur at various stages of the process, such as sorting by the generator, collection at curbside, and delivery to a central processing facility where final separation takes place. For example:

4.1.1 Recovery of compostable feedstock from leaves, brush and yard trimmings source-separated by the generator commonly includes the following: (1) accumulation in a separate container by the generator; (2) curbside and other collection and delivery to a central processing facility; (3) opening and removing plastic bags, if present; and (4) removing non-compostable and contaminant material that can accompany leaves, brush and yard trimmings (e.g., lumber scraps, toys, garden hoses, used vehicle oil and air filters, aerosol cans, bricks, concrete and rocks, and plastic bags).

4.1.2 Recovery of compostable feedstock from municipal solid waste is more complex. Two options exist. Option one relies on generators to presort the compostable and compost compatible material (based on a list of acceptable materials provided to them) into a container that is placed at curbside for collection. Collection can be either separate from other recyclable and waste material, or commingled with other waste and secondary resource material. Option two consists of curbside collection of the generator's accumulation of material mixed to some degree (as specified) with other household non-hazardous waste. Collection can be either separate from other recyclable material, or commingled. After delivery to a central processing facility, material received from both options are handled and processed in essentially the same manner, because both include not less than 3 to 11% "mistakes" that must be identified and removed from composting feedstock material. "Mistakes" can include recyclable material and material that can potentially cause compost quality problems.

4.1.3 Recovery includes: (1) collection and perhaps segregation of presorted material at curbside by a matching segregation at the side of the collection truck(s); (2) delivery to a central processing facility; (3) recovery of marketable material for recycling, e.g., old newsprint and corrugated cardboard, ferrous, aluminum, glass, etc.; (4) perhaps recovery of refuse-derived fuel; (5) separation of oversize material; (6) removal of non-compostable materials, e.g., stones and cigarette filter tips; (7) removal of other materials potentially detrimental to compost quality; and (8) recovery of compostable material. (Removal of some non-compostable material can take place after composting is complete, if the material being removed does not cause chemical contamination that prevents compliance with either regulatory or market quality standards.)

4.1.4 Separation at a central facility is generally by a combination of manual and mechanical methods, and is shown on the schematic diagram as separation of recyclables, refuse-derived fuel, and rejects disposal.

4.1.5 After recovery, the feedstock is ready to be prepared for composting. For two compostable feedstock sources, namely source-separated municipal solid waste and mixed municipal solid waste, the recovered feedstock is sometimes called mixed organic material.

4.2 *Step 2: Feedstock Preparation*—The feedstock preparation step may include physical processing and the addition of compost compatible materials which imparoves composting efficiency. Feedstock preparation may include the following:

4.2.1 Addition of carbon, nitrogen and phosphorus sources by mixing two or more feedstocks to establish a desired ratio of carbon to nitrogen, and perhaps carbon to phosphorus, to control odor generation, and to achieve market requirements for product stability.

4.2.2 Adjusting initial porosity to satisfy requirements for free airspace (to aid oxygen and gas exchange) and water-holding capacity (moisture). Reducing the particle size through shredding or tumbling increases surface area, helps creates uniform particle size, improves aeration (if particles aren't too small), and reduces volume. An alternative is particle size selection through screening. Adding a bulking agent, such as wood chips, to increase porosity and aid

air flow through the composting mass is normally necessary.

4.2.3 Adding water to the feedstock to adjust the moisture level to a desirable range.

4.2.4 Adding a small volume of compost that is in its peak heat thermophilic phase can 'jump start' the process by providing a cross-section of biological species for rapid colonization and biological activity.

4.2.5 Mixing to homogenize the feedstock, starter compost, and other additives, to distribute water uniformly, and to break down oversized particles of feedstock.

4.3 *Step 3: Composting*—Composting is a natural biological degradation process that is controlled and accelerated at a composting facility. Composting is the transformation of biologically decomposable material through a controlled process of biooxidation that results in the release of carbon dioxide, water, and minerals, and in the production of stabilized organic matter (compost or humus) that is biologically active. Because the biological process of composting follows a similar course regardless of the organic materials that are present in the feedstock, the schematic diagram (Fig. 01.02-A1) shows composting as a step common to each feedstock and one that may include the following:

4.3.1 Aeration to maintain optimum conditions for aerobic microbial activity, to supply oxygen, to buffer pH and immobilize ammonium, to remove heat, to remove carbon dioxide, to remove moisture, to strip volatile compounds, and to avoid anaerobic conditions and odor generation.

4.3.2 Temperature control to reduce pathogens to background levels and destroy weed seeds, and to maximize the rate of decomposition both during the high temperature thermophilic phase and afterward during the mesophilic phase.

4.3.3 Addition of make-up water to maintain moisture content for aerobic conditions, and to maximize organic decomposition.

4.3.4 Mechanical agitation or turning to thoroughly mix make-up water uniformly throughout the decomposing mass, to break up air channels and clumps, to prevent fly reproduction, and to produce a uniform product.

4.4 *Step 4: Odor Treatment*—Process air is captured and routed through a biofilter or other positive treatment method. In a biofilter, operating conditions must favor porosity, ample oxygen and moisture to ensure conditions highly favorable to active microbial populations that are not associated with odor-causing compounds such as hydrogen sulfide, volatile fatty acids and ammonia.

4.5 *Step 5: Compost Curing*—Customers that require a mature product may specify a greater degree of product biological stability. Compost curing is the last stage of composting that occurs after much of the readily metabolized material has been decomposed. Compost curing provides for additional stabilization, and allows further decomposition of cellulose and lignin. Cured compost is a highly stabilized product that results from exposing compost to a prolonged period of humification and mineralization, ranging from six to eight months.

4.6 *Step 6: Compost Screening and Refining*—Screening is necessary to remove contaminants such as oversized material, stones, metal fragments, glass, film plastic, hard plastic, and sharps. Residue from screening and refining can be recycled, or it might be disposed of at an incinerator or landfill. Screening also enables size classification to suit customer needs. Some customers may require that essentially all man-made inert material is removed from compost to enhance its aesthetic acceptability. Further refining can remove small stones, glass, metal fragments, hard plastic, film plastic, and sharps. This step typically follows the compost curing step so as to retain bulking material in the compost as long as possible as an aid to aeration

4.7 *Compliance with Compost Safety Standards*—Standards to protect public health, safety, and the environment typically focus on the content of trace metals and pathogens in compost. However, soluble salts, pH, man-made inerts, film plastic and organics may also be the focus of compliance standards in some cases. Verification is by standard methods for sampling, preparation and analysis. If a compost product meets the minimum standards for safety, the product is a "General Use Compost" and may be distributed for use as a soil amendment. Although a product may meet minimum requirements for public health, safety, and the environment, it may not, however, suit a particular customer's aesthetic requirements for example, or a customer's requirements for stability or for nutrient value. Product marketing issues will dictate the extent of curing, refining, and amending necessary to meet customers' needs. If the product does not meet the suggested minimum standards, the product may be reprocessed to meet the standards, or may be marketed as a "Designated Use Compost", with specific restrictions on its use appropriate to its characteristics. For example:

4.7.1 A compost which does not meet the minimum standards for compost stability could be (1) composted for another week or more until it is sufficiently stable to meet the stability standard, or (2) used in an application where its relatively low stability would not be harmful.

4.7.2 A compost which contains more film plastic than the standard could be (1) refined to remove the film plastic, or (2) marketed for restricted use where the film plastic content would be acceptable.

4.8 The Composting Schematic Diagram (The Process Model, Fig 01.02-A3) was developed as an aid to a broad understanding of process steps in the manufacture of compost suitable for general distribution or for designated uses.

4.8.1 As noted previously, a product that complies with regulatory standards for safety is suitable for distribution and use as a soil amendment. It is classified "General Use Compost".

4.8.2 "Designated Use Compost", on the other hand, is the classification that does not comply with safety standards and its distribution and use is subject to regulatory control. The authors elected not to show market segments for this class of compost on the model schematic, because use is restricted by local or state regulation. Uses are potentially numerous. It might be used, for example, as landfill daily cover, or refined for land reclamation, and/or cured for other designated, restricted markets.

4.8.3 *General Use Compost* classification is shown as the source of supply for three grades of compost, namely *Raw Compost*, *Refined Compost*, and *Cured Compost*. These three grades of compost are manufactured for reliable and sustainable end markets. Each of the three grades of compost may be amended with supplemental material (amendments) added during composting or to compost, to provide attributes required by customers for certain compost products, such as product bulk, product nutrient value, product pH, and soils blend. Marketing consultants have defined a dozen or more major market segments. Marketers supply compost to many segments and more are being developed.

4.8.3.1 *Raw Compost* is generally suitable for use as landfill cover material and as surface mine reclamation material. It may be suitable for other additional uses.

4.8.3.2 *Refined Compost* is generally suitable for field nursery use, sod production, silviculture, agriculture, commercial landscaping, and specialty applications such as erosion control and for biofilters. It may be suitable for other additional uses.

4.8.3.3 *Cured Compost* may generally be used for high-end landscaping, delivered topsoil, bagged/retail markets, container nurseries, and specialty applications, (e.g., wetlands redevelopment, etc.).

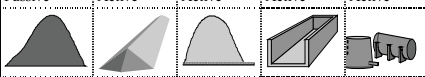
01.03 COMPOSTING TECHNOLOGY GROUPS

01.03-A OPERATION CHARACTERISTICS

1. Technologies

1.1 Technologies used for composting cover a broad spectrum of options. The composting industry recognizes five groups of composting technologies, as shown in the Table 01.03-A1.

Table 01.03-A1 Composting Technology Groups.

Management Strategies *Step 3:* Composting	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Weather Protection	Open	Open	Covered	Covered	Covered
Pile Configuration	Piles	Windrows	Piles and Tunnels	Windrows Trenches Beds and Bays	Tunnel and Vessel Systems
Process	Passive	Active	Active	Active	Active
Management of Key Process Variables					
▪ Pile	Undisturbed	Turned	Static Structure	Turned	Turned
▪ Feedstock nutrient balance	Unmanaged	Initial C:N ratio set	Initial C:N ratio set	Initial C:N ratio set	Initial C:N ratio set
▪ Pile Oxygen and pH	Unmanaged	Convective Aeration	Forced Aeration	Mechanical Aeration	Forced Aeration
▪ Pile Moisture	Unmanaged	Mix in Make-up	Mix in Make-up	Mix in Make-up	Mix in Make-up
▪ Pile Temperature	Unmanaged	Turning Control	Blower Control	Blower Control	Blower Control
▪ Retention Time	12-14 months	2-12 months	2-6 months	2-5 months	2-4 months

1.1.1 The five [5] technology categories are based on the predominant composting unit operation characteristics, a technology classification system devised and peer-reviewed for the *Composting Facility Operating Guide,* US Composting Council, 1994 (http://compostingcouncil.org).

1.2 The composting industry categorization of technologies is generic. Specific technology suppliers offer variations within a technology group. A single technology should offer a combination of alternatives that will address the seven steps of the composting process.

1.3 Sampling and testing plans must be designed to suit the specific approach used in each composting project.

1.4 Sampling and testing marketable product provides the data needed to demonstrate compliance with environmental standards and to provide users with information needed to plan for proper application of compost products.

2. Composting Feedstocks

2.1 Six types of source materials commonly composted:

2.1.1 *Food Processing Residuals*—compostable material remaining after fruit, vegetables, grains, nuts, and meat are processed for consumption.

2.1.2 *Manure and Agricultural By-Products*—generated at racetracks, feedlot and other animal feeding operations, farms, nurseries, and greenhouses. Compost produced from residuals generated from farms, nurseries, and greenhouses can be readily recycled into those operations. However, huge quantities of manure generated at racetracks, feedlots, and swine and poultry confinement facilities can pose a significant challenge to facility operators.

2.1.3 *Forestry and Forest Product Residuals*—includes bark and sawdust, and fiber fines residue and biosolids generated by the papermaking process. Bark and sawdust can be used in the composting industry as a carbon source with other feedstock material or as a bulking material to increase porosity of the feedstock mix.

2.1.4 *Biosolids, or Sewage Sludge*—the solid material generated by the biological treatment of sewage at a wastewater treatment plant. In addition to being composted, sewage sludge can be recycled for beneficial use by direct application onto land as a fertilizer.

2.1.5 *Leaves, Brush and Yard Trimmings (Yard Waste)*—typically consists of leaves, brush, grass clippings, plant trimmings, and plant remains. Historically this material has been collected with municipal solid waste and incinerated or landfilled. Many communities have restricted incineration and landfilling of these materials. Instead, they are separately collected and recycled for beneficial use by directing it to a mulching or composting facility.

2.1.6 *Source Separated Organic Waste (SSOW)*—consists of the compostable and composting compatible fraction of municipal solid waste, accumulated and presorted by the generator, and collected separately from household hazardous material and sometimes non-compostable material. The compostable and composting compatible fraction can be directed to a central composting facility. For example, a compostable organics collection program that relies on source separation by the generator could include leaves, brush and yard trimmings, food scraps, wet and soiled paper, diapers and sanitary products, pet waste, and dry paper packaging that is not recycled because of weak or nonexistent markets. Source-separated organic waste includes residential, institutional and commercial

sources, and can include the domestic portion of the industrial solid waste stream.

2.1.7 *Mixed Municipal Solid Waste (MSW)*—includes various discards from residential, commercial, and institutional sources that are commonly taken to incinerators or landfills. The largest components of mixed municipal solid waste are typically paper and paper products, leaves, brush and yard trimmings, wood, food scraps, glass, plastics, and metals. The composition of mixed MSW varies depending on the characteristics of the waste generators in the service area, but usually from about 50% to 65% is compostable when recovered by separation at a central facility. Mixed municipal solid waste will contain relatively fewer recyclables and a relatively higher fraction of compostable material when an aggressive source-separated recycling collection program operates in conjunction with mixed municipal solid waste collection.

2.2 Market attributes of finished compost product are influenced both by the feedstock used and by composting process control.

2.3 Sampling and testing plans must be designed to suit the specific feedstocks used in each composting project.

3. Compost Product Overview

3.1 A variety of value-added products are developed by processors (Fig 01.02-A2 Composting Products Model).

3.1.1 *Step 1*—Feedstock Recovery involves an inspection of materials received at the tip floor or receiving area to removed unwanted items from the feedstock. This step may also be used to gather items that have commercial value in the traditional recycle markets, such as ferrous, plastics, and clean paper. Equipment and labor for sorting is often required and returns should offset costs.

3.1.2 *Step 2*—Feedstock Preparation involves establishing the initial porosity and degradability, i.e., C:N and C:P ratios, moisture content, etc.

3.1.3 *Step 3*—Thermophilic Composting reduces pathogens, which is a fundamental requirement for feedstock processing and destroys most weed seeds. At this point, markets may be able to accept the material even though it is neither biologically stable nor free of organic phytotoxins that inhibit seed germination and plant growth. (e.g., land applied for sufficient time to reach a level of stability and maturity before planting that benefits crop growth and soil conservation).

3.1.4 *Step 5*—Compost Curing increases biological stability, may further reduce pathogens, and with aeration eliminates organic phytotoxins. The degree of curing should match the intended use of the product. A bagged product will require a high degree of curing because of its potential use as potting soil and garden bed amendment. Product that will be sold in bulk for direct application for agricultural, landscape, roadside, or reclamation settings may not require as much curing as compost used a potting soil.

3.1.5 *Step 6*—Compost Screening and Refining removes oversized material and other unwanted material, and can provide the particle size and texture of product for particular end use requirements. Screening and refining equipment is an investment that should increase product revenues.

3.1.6 *Step 7*—Compost Storing and Packaging deals with seasonal demand patterns that don't match feedstock availability patterns and may generally be inevitable. No degradation of product should be allowed, and if storage is inevitable it can be exploited to add still more value for the highest markets of all that include bagged and amended products.

Test Method: Field Sampling of Compost Materials. Five Protocols						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
02.01-A	02.01-A	02.01-A	02.01-A	02.01-A	02.01-A	02.01-A	02.01-A	02.01-A
02.01-B	02.01-B	02.01-B	02.01-B	02.01-B	02.01-B	02.01-B	02.01-B	02.01-B
02.01-C	02.01-C							
		02.01-D	02.01-D	02.01-D	02.01-D	02.01-D	02.01-D	02.01-D
02.01-E	02.01-E		02.01-E			02.01-E	02.01-E	02.01-E

02.01 FIELD SAMPLING OF COMPOST MATERIALS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org/addenda.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Source

1.1 This section covers sampling procedures for compost and composting feedstock.

1.1.1 *Method 02.01-A Compost Sampling Principles and Practices* adapted from sampling procedure documents provided by Dr. William F. Brinton, Woods End Research Laboratory, 1996.

1.1.2 *Method 02.01-B Selection of Sampling Locations for Windrows and Piles.*

1.1.3 *Method 02.01-C Sampling Plan for Composted Material*—adapted from the US EPA's Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846, Third Edition, September, 1986. Consideration and importance was placed on sampling composted solid waste rather than sampling sediments, sludges, or soils for waste analysis. Most information remained unchanged. The majority of the information on sampling was taken from Chapter Nine, Volume II of the U.S. EPA Solid Waste - 846 Manual.

1.1.4 *Method 02.01-D Composting Feedstock Material Sampling Strategies*.

1.1.5 *Method 02.01-E Data Quality Management and Sample Chain of Custody.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

ASTM D 5231-92, Determination of the Composition of Unprocessed Municipal Waste. *In* Annual Book of ASTM Standards, Vol. 04.08

ASTM D 4547-91, Sampling Waste and Soils for Volatile Organics. *In* Annual Book of ASTM Standards, Vol. 04.08

A Plain English Guide to the EPA Part 503 Biosolids Rule. US EPA Office of Wastewater Management. EPA/832/R-93/003, September 1994.

Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. US EPA SW-846. 3rd Edition, September, 1986.

Statistical Quality Control Handbook. Western Electric Company, Inc. 2nd Edition. 1958.

3. Terminology

3.1 *aliquot, n*—a sub-sample of a material prepared for, and subjected to laboratory analysis. A sub-sample size smaller than 1 g may be used to represent more than 1000 kg of compost.

3.2 *attribute verification, n*—a laboratory protocol that includes standard reference materials, checks and blanks to validate analytical determinations.

3.3 *confidence interval, n*—a statistical range with a specified probability that a given parameter lies within that range. The magnitude of the range increases as the specified probability is increased.

3.4 *process monitoring, n*—samples collected at predetermined intervals within the composting process to track the targeted changes in biological, chemical and physical characteristics; key process variables in compost piles that should be monitored include porosity, oxygen percent, moisture percent, temperature, retention time or age.

3.5 *process variability, n*—deviations from optimal management procedures of compost production that

may induce deviations in the desired result and sub-optimal finished compost.

3.6 *product variability, n*—heterogeneity of the chemical, biological and physical characteristics of a compost product attributable to both the composting process and the heterogeneity of input feedstocks.

3.7 *representative sample, n*—a sample that accurately reflects the average chemical, biological and physical characteristics of interest from the source of feedstock, bulk material or compost batch in question.

3.8 *sample collection frequency, n*—retrieval of representative samples at intervals that accurately represent the status within the process step of interest for the bulk of compost in question or batch of concern.

3.9 *statistical validity, n*—determinations made from a sample that accurately represent the average characteristics of the compost of interest.

4. Sampling Collection and the Composting Process

4.1 A generalized model developed to represent the aerobic composting process is presented in Fig 02.01-1 Composting Unit Operations Model.

4.1.1 Market attribute analytical values for a finished compost vary according to the type or blend of composting feedstocks and composting process. Value-added compost products are illustrated in Chapter 01.00 Fig 01.02-A2 Composting Products Model. Sampling and testing plans must be designed to suit the feedstock used in composting, the specific approach to feedstock preparation and composting process management in each composting project, and specifically for each finished product.

4.2 *Selection of Sampling Method:*

4.2.1 *Feedstock Sampling Location*—The sampling location for composting feedstock is after feedstock recovery (step 1) has been completed. Feedstock sampling is performed after routine removal of recyclable and/or problem materials. Samples should be taken before feedstock preparation (step 2), i.e., before shredding or size reduction, and before supplemental nutrients, bulking agents or water have been added. The facility operators can provide the best information for the locations to obtain feedstock samples.

NOTE 1—Once the feedstock preparation, (step 2 of the composting process model), is completed, the actual composting process begins with the material placed in piles, windrows or reaction vessels for composting.

4.2.2 *Prepared Feedstock Sampling*—Samples should be taken after feedstock preparation before composting. Facility operators can provide the best information for the locations to obtain feedstock samples.

4.2.3 *Composting and Compost Curing Process Control Sampling Locations*—The sampling location for process monitoring during composting, step 3, and compost curing, step 6, is indicated in Fig 02.01-B1 Hypothetical Sample Collection Pattern from a Compost Pile.

4.2.4 *Finished Compost Sampling Locations*—Finished compost is expected to match the needs of the customers, and may be obtained from step 3, Composting; step 5, Compost Curing; step 6, Compost Screening and Refining; and step 7, Compost Storing and Packaging as indicated in Chapter 01.00 Fig 01.02-A2 Composting Products Model. Finished compost samples are taken from the actual product that is released for distribution to an end-user.

5. Summary of Methods

5.1 *Method 02.01-A Compost Sampling Principles and Practices*—Review of sampling design schemes adapted from sampling procedure documents provided by Dr. William F. Brinton, Woods End Research Laboratory, Inc.

5.2 *Method 02.01-B Selection of Sampling Locations for Windrows and Piles*—Descriptions of sample collection as sets of compost sub-samples collected and combined to represent the average chemical, physical and biological characteristics of the compost material for a batch windrow or pile of cured or curing compost.

5.3 *Method 02.01-C Sampling Plan for Composted Material*—Review of US EPA SW-846 sampling plan guidelines and statistical procedures for estimating required minimum number of samples.

5.4 *Method 02.01-D Composting Feedstock Material Sampling Strategies*—A representative sample of feedstock is collected to identify its chemical and physical characteristics.

5.5 *Method 02.01-E Data Quality Management and Sample Chain of Custody*—Consideration for third-party sample collection and preparation. Also, an example form and description of the parameters needed for a chain of custody report.

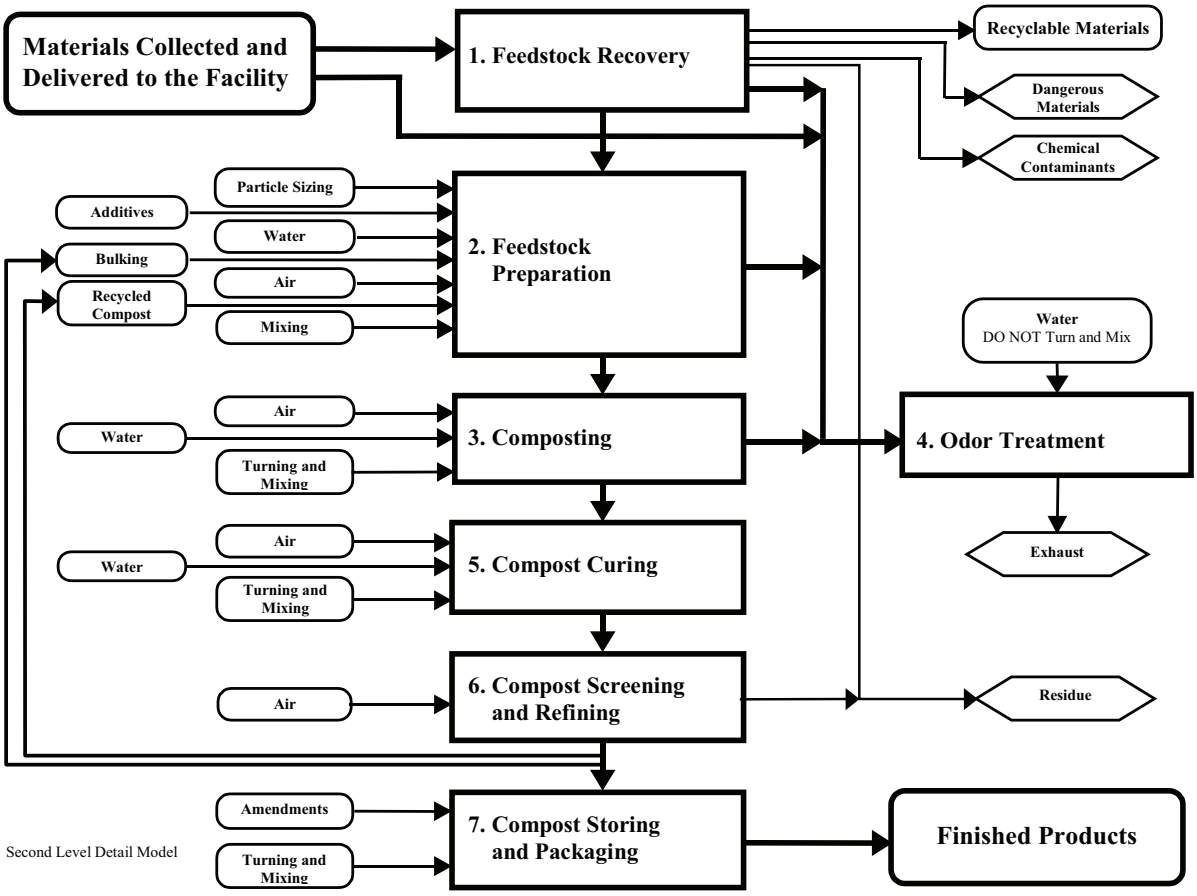


Fig 02.01-1 Composting Unit Operations Model.

6. Significance and Use

6.1 *Method 02.01-A Compost Sampling Principles and Practices*—Source of general guidelines and considerations needed to develop an appropriate compost sampling plan.

6.2 *Method 02.01-B Compost Material Sampling Strategies*—A general guide for compost sample collection and preservation from compost curing piles.

6.3 *Method 02.01-C Sampling Plan for Composted Material (from SW-846 Chapter Nine, part 1)*—The initial, and perhaps most critical element in a program designed to evaluate the physical, chemical and biological properties of a compost is the plan for sampling the material in question. It is understandable that analytical studies, with their sophisticated instrumentation and high cost, are often perceived as the dominant element in a characterization program. Yet, despite that sophistication and high cost, analytical data generated by a scientifically defective sampling plan have limited utility.

6.4 *Method 02.01-D Composting Feedstock Material Sampling Strategies*—A general guide for feedstock sample collection. Specific methods should be modified for differing feedstock materials.

6.5 *Method 02.01-E Data Quality Management and Sample Chain of Custody*—A method of tracking a collected sample from date, time and location of sampling through completion of laboratory analysis.

7. Interference and Limitations

7.1 Analytical error associated with sampling and handling is compounded when multiple properties with conflicting sampling needs are measured from the same sample. For example, it is a good idea to subdivide and remix samples repeatedly if mineral and metal tests are being performed. This improves homogeneity and reduces sample variance. Unfortunately, this same method induces excessive volatilization of some of the compounds, and causes microbial cross-contamination. Therefore, the sampling plan must specify a separate sampling and handling scheme for each test parameter that requires special sampling.

7.2 *Method 02.01-B Compost Material Sampling Strategies*—As compost heterogeneity increases, the number of sub-samples should be increased. If insufficient numbers of samples are collected, analytical results will not represent the compost in question.

7.2.1 Moisture loss or gain during sample handling and splitting may become significant. It is therefore necessary to mix and split a sample under sheltered conditions, such as inside a building where wind, temperature and sunlight or precipitation will not distort the compost moisture.

7.3 *Method 02.01-C Sampling Plan for Composted Material*—Knowledge of or access to statistical procedures is required.

7.4 *Method 02.01-D Composting Feedstock Material Sampling Strategies*—Sample heterogeneity of feedstock may be much higher than that of the finished composted product. It is crucial that all sampling plan collection procedures are followed to maximize the reliability and accuracy of the feedstock sample analytical results.

7.4.1 Moisture loss or gain during sample handling and splitting may become significant. It is therefore necessary to mix and split a sample under sheltered conditions, such as inside a building where wind, temperature and sunlight or precipitation will not distort the feedstock moisture.

8. Sample Handling

8.1 Collect samples from areas of the compost pile that are representative of the general appearance, and avoid collecting atypically moist samples (> 60% moisture, wet basis). If balls form during the process of blending and mixing of point-samples, the compost sample is too wet. Excessively moist compost will cause unreliable physical and biological evaluation.

8.2 For most feedstock or compost samples, use containers made of stainless steel, plastic, glass or Teflon. These materials will not change compost chemical quality. Laboratories provide advice on appropriate sample containers, preservatives and shipping instructions when requested.

8.3 A representative compost sample must be collected from appropriate sampling locations and consist of no less than 15 point-samples. Sampling locations along the perimeter of the compost pile where compost point-samples will be extracted and vertical distances from the ground or composting pad surface shall be determined at random, and shall be representative of the compost on the site.

8.3.1 Determine the number and types of sampling and shipping containers to be used. The composite sample is placed in a sanitized container and thoroughly mixed. Follow proper quality assurance/quality control procedures for sample preservation, storage, transportation and transfer. Sample the cured compost and aliquot 12 L (3 gal) sub-samples from the composite sample and place in a sanitized plastic container and seal.

8.3.2 Utilize the Student's “t”-test with a confidence interval of 80% to statistically analyze the test data. Refer to TMECC 02.01-A, paragraph 9.10 *Sampling Intervals* for guidance in determining sample collection frequency.

8.4 *Test Methods versus Sampling Methods*—The laboratory test method and analytical parameter of interest dictate the method of sample collection, type of container for shipping and storage of samples and sample handling procedures required. Table 02.01-1 provides a partial list of analytical traits that are affected by sample collection and handling. In general, volatile compounds and elements, physical bulk factors and microbiological samples require special considerations when developing the sampling plan.

Table 02.01-1 Partial list of test parameters that require special sampling and handling considerations.

Test Parameter	*Principle Constraint*	*Associated Error*	*Alteration of Sampling for Corrective Action*
Total-N	Volatilization loss of NH_3 during sample handling	Underestimation of total N and volatile N	Place in container quickly with minimal stirring
Volatile fatty acids (VFA)	Volatilization loss of VFA during sample handling	Underestimation of VFA content	Place in container quickly with minimal stirring
Microbiology (pathogens)	Contamination from tools, buckets, air	Over or under estimation of pathogens	Use only clean, sterile containers and implements
Bulk Density	Excess sample moisture	Overestimation of volume/weight	Take large, oversized samples

8.4.1 In each case the determination for a trait of interest can be changed adversely by improper sample collection and handling, and consequently lead to erroneous conclusions. Analytical precision or relative variability may not be affected by inappropriate sampling, but accuracy of the expected determination may be biased and incorrect.

8.5 *Containers, Post-Sample Handling*—For each type of parameter measured after sampling specific containers and holding times should be observed prior

to and during transport to a laboratory (see Tables 02.01-2 through 02.01-6). Use multiple containers to preserve sample integrity as necessary.

8.5.1 Despite the wide variation in sample holding times and condition requirements, all compost samples targeted for general testing should be chilled immediately upon collection and preparation. Refer to Tables 02.01-2 through 02.01-6 to find the most appropriate storage temperature for each test parameter of interest.

8.5.2 When plastic containers are acceptable, use double Ziploc®-type 4-8 L (1-2 gal) bags marked on the exterior with a marking pen with insoluble ink, and placed with several cool-packs in a large polystyrene cooler or similar insulated container.

8.5.3 Ship the samples to the laboratory for delivery within 24 h or less. Request that the laboratory staff store samples at 4°C when delays in lab preparation are anticipated.

8.5.4 Collection and storage of samples for organic compound analysis - polychlorinated biphenyls (PCBs), polynuclear aromatic hydrocarbons (PAHs) or volatile fatty acids (VFAs) - require glass containers with Teflon lids, or exclusively Teflon containers. Sample containers should be filled to overflowing with material to minimize airspace in the container and reduce volatilization of organic compounds during storage.

8.5.5 Include proper *Chain-of-Custody* information: date, time, name of the sampling entity and name individual responsible for sample. Refer to *Method 02.01-E Data Quality Management and Sample Chain of Custody* for an example form and description of parameters needed to complete a chain of custody report.

Table 02.01-2 *Physical Parameters:* Sampling containers and conditions for compost and source ingredient testing.

Test Parameter of Interest	*Container*	*Conditions*	*Maximum Holding Time Allowed in Lab*
Bulk Density, Hydraulic Conductivity, Porosity, Water Holding Capacity	P, G	4°C	7 d
Temperature	*NA*	*NA*	Immediate, no delay
Total Solids	P, G	4°C	24 h

NOTE 2—P=Plastic; G=Glass

Table 02.01-3 *Organic and Biological Properties:* Sampling containers and conditions for compost and source ingredient testing.

Test Parameter of Interest	*Container*	*Conditions*	*Maximum Holding Time Allowed in Lab*
Respirometry	P, G	4°C	24 h
Organic Carbon	P, G	4°C	14 d
Volatile Fatty Acids	G (2 L CWM)	4°C	14 d
Volatile Solids	P, G	4°C	14 d

NOTE 3—P=Plastic; G=Glass

Table 02.01-4 *Chemical Parameters:* Sampling containers and conditions for compost and source ingredient testing.

Test Parameter of Interest	*Container*	*Conditions*	*Maximum Holding Time Allowed in Lab*
Acidity/Alkalinity (pH), Electrical Conductivity, Kjeldahl Nitrogen, Nitrate Nitrogen (NO_3-N), Nitrite Nitrogen (NO_2-N), Ammonia Nitrogen and Ammonium Nitrogen (NH_3-N, NH_4-N), Sulfide	P, G	4°C	48 h
All other Metals	P, G	4°C	6 months
Chloride, Sulfate	P, G	4°C	28 d
Chromium VI	P, G	4°C	24 h
Mercury	P, G	4°C	28 d

NOTE 4—P=Plastic; G=Glass

Table 02.01-5 *Pathogens:* Sampling containers and conditions for compost and source ingredient testing.

Test Parameter of Interest	*Container*	*Conditions*	*Maximum Holding Time Allowed in Lab*
Enteric Virus	G	-70°C	> 8 h
Enteric Virus	SP, G	4°C	8 h
Coliforms and other bacteria	SP, G	4°C	48 h
Helminth Ova	SP, G	4°C	1 month

NOTE 5—SP=Sterilized Polypropylene; G= Sterilized Glass

Table 02.01-6 *Synthetic Organic Compounds:* Sampling containers and conditions for compost and source ingredient testing.

Test Parameter of Interest	*Container*	*Conditions*	*Maximum Holding Time Allowed in Lab*
Chlorinated Herbicides, and Chlorinated Hydrocarbons, PCB	G, Teflon lined cap (2-$^1/_2$ L.A.J.)	4°C	7 d until extraction
Chlorinated Pesticides	16 oz B.R. (2-$^1/_2$ L.A.J.)	4°C	7 d until extraction
Dioxins & Furans, Nitroaromatics and Isophorone, and Polycyclic Aromatic Hydrocarbons, PAH	G, Teflon lined cap (2-$^1/_2$ L.A.J.)	4°C store in dark	7 d until extraction
Phthalate esters	G, Teflon lined cap	4°C	7 d until extraction
Purgeable aromatic hydrocarbons	G, Teflon lined septum (40-mL Glass V)	4°C	14 d prior lab testing
Semi-Volatile Organics	G, Teflon-lined Septum (2.5-L Jug)	4°C	7 d
TCLP Sample	G, Teflon-lined Septum (2.5-L Jug)	4°C	7 d until extraction
Volatile Organic Compounds (VOC)	G, Teflon lined septum (40-mL Glass V)	4°C	14 d preserved in HCl†

NOTE 6—P=Plastic; G=Glass, HDPE=High Density Polyethylene

†—Evaluation data is being sought to confirm this requirement for curing and finished composts.

Test Method: Compost Sampling Principles and Practices							**Units:** *NA*	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
02.01-A	02.01-A	02.01-A	02.01-A	02.01-A	02.01-A	02.01-A	02.01-A	02.01-A

02.01-A COMPOST SAMPLING PRINCIPLES AND PRACTICES

COMMENT—This section was adapted from sampling procedure documents provided by Dr. William F. Brinton, Woods End Research Laboratory, 1996.

9. Justification for Compost Sampling

9.1 Sampling of compost and compost products is an essential aspect of process monitoring, quality control, marketing and labeling, and regulatory compliance. Like other functions of site management, sample collection involves carefully planned and often labor intensive activities. Four common reasons for compost sampling are described:

9.1.1 *Ingredient Analysis*—basic data on source ingredients are needed for the design of a composting process or identification of an optimal composting feedstock recipe.

9.1.2 *Process Design and Monitoring*—composting process evaluation requires information on material characteristics and process benchmarks. Specific sample collection protocol is designed for each parameter of interest.

9.1.3 *Marketing and Labeling*—specification sheets or product labels for compost are needed to compare product with others in the marketplace.

9.1.4 *Regulatory Compliance*—compost process and product requires periodic testing for compliance with specified traits including certain metals, pathogens, stability and maturity.

9.2 *Use of Sampling Data*—Sampling decisions require an understanding of the need for data collection, specifically how to sample and when to collect samples. The sampling decision tree presented in Fig 02.01-A1 illustrates a decision process to assist in the development of proper sample collection methods, to identify sampling interval and sample size, and the end use of sample data. When regulations do not apply, as is the case for recipe formulation, process monitoring for quality assurance (QA) and internal quality control (QC), it is important to clearly understand the intended use of the data and to determine the appropriate sampling procedures. For example, if C:N ratio interpretation is considered very important, then very low variations in sample carbon and nitrogen determinations become a major consideration and a sample collection process must be designed to support to this requirement.

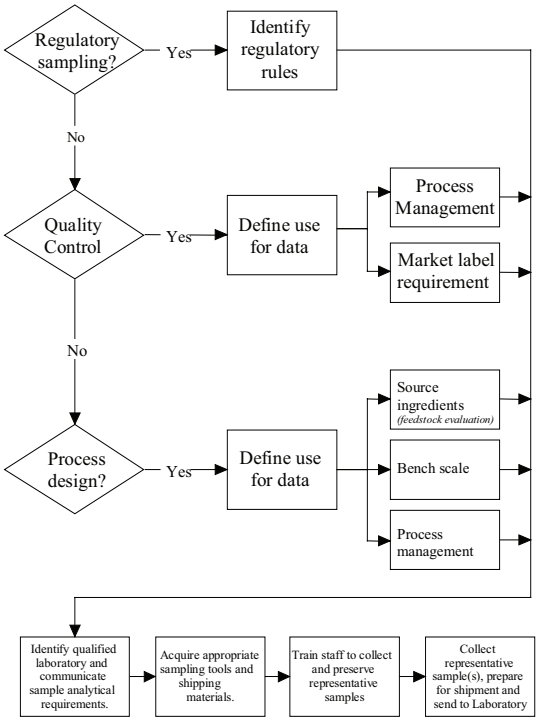


Fig 02.01-A1 Compost sampling decision tree, overview of sampling objectives.

9.3 *Types of Sampling*—Two types of sample collection are used: point-sampling and composite-sampling:

9.3.1 *point sampling*—site-specific sample collection from within the general mass is used to identify and quantify points of extreme variability, hot spots or problem zones. Point-sampling alone should not be used unless special conditions exist.

9.3.2 *composite-sampling*—a single sample for laboratory analysis composed of multiple, well-blended point- or sub-samples uniformly distributed throughout the entire volume that, after mixing, accurately represents an average or median value of the property or trait of interest for a batch or general mass. Properly implemented composite sampling is preferable for most sampling plans because it provides a reliable estimate

of the average or median property or trait of a batch or segment of a continuous stream, rather than a specific spot trait.

9.3.2.1 *stratified sampling*—a modified composite sampling scheme is used to document gradients and define heterogeneity as a function of position within the bulk or general mass of sampled material, where the general mass is subdivided into separate zones and a series of point-samples are collected and composited within each zone. Stratified sampling should be used when heterogeneity of compost is unknown and when regulatory constraints require knowledge of the relative spatial and temporal variability. This is most often based upon the standard deviation and mean; refer to Method 02.01-B for equations applied in calculations for approximating the required number of sub-samples to accurately estimate the average value for the parameter or trait of interest.

9.3.2.2 *interval sampling*—sampling from moving conveyor belts.

9.4 *Sampling Plan*—The constraints of the material and the composting technology must be considered when an optimal sampling plan is designed. Combinations of composite and point sampling are illustrated within the four sampling schemes presented in Fig 02.01-A2. The sampling scheme selected must address limitations of the selected test parameter and should not distort the analytical result.

9.4.1 Stratified sampling (Scenario A, Fig 02.01-A2) is used to determine variability, profile gradients and spatial uniformity characteristics. In most cases, composite sampling (Scenario B, Fig 02.01-A2) is satisfactory when the amount of variability within the mass is known to be insignificant. It involves combining several representative sub-samples into one composite sample that is then thoroughly mixed, then split for shipment to the laboratory. Area or batch sampling (Scenario C, Fig 02.01-A2) and single grab- or point-sampling (Scenario D, Fig 02.01-A2) are for special cases where one sample is collected at one location. Area or batch sampling is typified by a whole mass collected as one sample unit. This method is most appropriate when moving the mass from a vessel to a curing pile. A single point-sample does not provide a representative sample for the bulk mass. Batch sampling and point sampling should be employed to characterize an obvious or potential anomaly at one specific point, time or location within a process. A good example of a single point sample to detect anomalies is shown as X in Fig. 02.01-A2 D, a location referred to as the "toe" of a static aerated pile, and one which is vulnerable to suboptimal temperatures needed to achieve pathogen reduction. For this reason, it is sometimes specifically included to verify pathogen content of compost that has finished the thermophilic phase.

9.5 *Importance of Representative Sampling*—A representative sample defines a material's average characteristic, typical for the entire material being sampled. Under virtually all composting conditions, the mass of compost material is large and heterogeneous. A representative sample of compost is not easily obtained; and sampling must be repeated over time to compensate for naturally high variations. Under proper management and as compost-curing advances, variability within a curing pile or windrow will decrease.

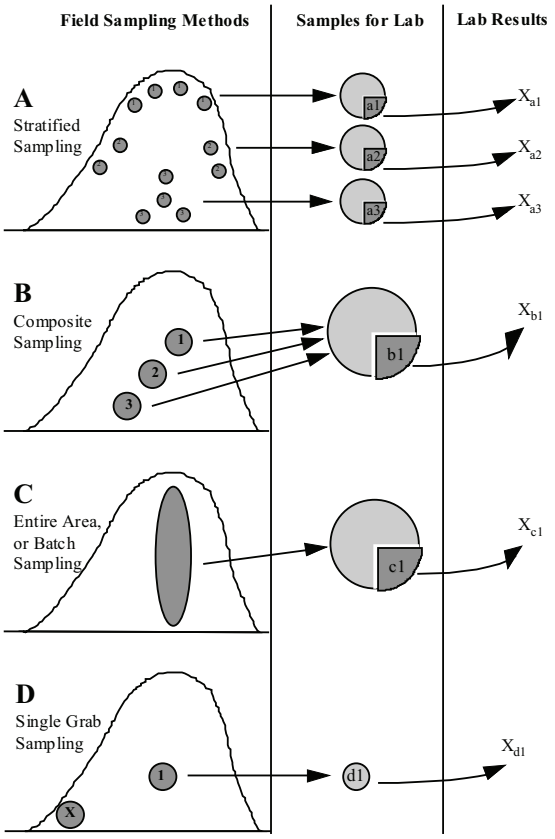


Fig 02.01-A2 The sampling schematic.

9.6 *Variables that Compromise Quality of Sampling*—Sample collection technique and variability of compost and cured compost affect the relative accuracy of sampling and the reliability of laboratory analytical determinations. Failure to adjust sampling protocols according to the nature and source of variations may invalidate test results and lead to inappropriate management or marketing decisions.

9.6.1 *Bias Introduced by the Sampler*—Inaccurate sample collection is often due to systematic or intentionally selective sampling introduced by the sampler. Significant error will result from attempts by the sample collector to counteract perceived variability. Examples include avoiding the collection of sub-

samples from wet pockets or systematically excluding large particles from the composite sample. **Deliberate bias results from an attempt by the sampler to prepare samples that appear superior in a perceived physical trait that does not actually represent the bulk or batch of interest.**

9.6.2 *Sample Heterogeneity*—The following are key sources of non-uniformity that can give rise to significant sampling errors.

9.6.2.1 *Sub-sample size* affects sampling accuracy. In general, a representative composite sample contains large (> 1000 cm^3) and plentiful sub-samples (>15 samples).

9.6.2.2 *Complete and thorough mixing* throughout the composting process improves the quality and ease of sampling. Poor initial mixing effects variability of the parameters throughout the composting process. Repeated use of turning machinery during composting improves homogeneity. However, within days or even hours after turning, mixing or re-piling, the composting mass may develop gradients of stability, moisture, bacteria and ammonia. When pre-mixing, blending or turning are not employed, as in static pile composting or compost curing, the sampling plan should include more sub-samples per composite sample to compensate for inherently high variability within the mass.

9.6.2.3 *Soil and stones* are frequently picked up during routine compost production operations. These pose problems for good sampling. In some cases, the sampler may bias the sample by deliberately excluding gravel and stones present in a compost (soil can not be easily seen). On the other hand, a laboratory that receives a sample containing stones or small gravel may not sub-sample, pre-screen, and grind, resulting in variable results. Staff responsible for sampling must correctly diagnose the situation and advise the analytical laboratory about it. In some cases, laboratories must issue disclaimers about their own sub-sampling technique.

9.6.2.4 *Foreign and non-compostable matter* almost invariability poses problems to the sampler, and also the laboratory. This is most likely the case with municipal solid waste (MSW) and certain industrial by-products where large and variable amounts of such substances are present. The best approach is to take large sub-samples and blend frequently before removing the final sub-sample for examination or testing. There is presently no generally accepted or standard practice for gauging the minimum sample size required in such situations.

9.6.2.5 *Varying particle size* is one of the most common sources of sample variability. For example, a composting feedstock mix may have exactly 27% wood chips, but inability to sub-sample adequately could result in finding anywhere from 11 to 38% wood chips. The error introduced to C:N values for samples of this range is significant.

9.6.2.6 *Layering, compaction and gradients* of composts arise as a result of inadequate initial mixing, infrequent or excessive turning/mixing during feedstock preparation, or during the composting process because of equipment/ventilation actions such as inappropriate selection and use of bulking materials. Any one or more of these can easily confound sampling attempts.

9.7 *Sampling Practice*—Sampling begins with the decision to evaluate materials and proceeds to determining how and in what time frame the sample is needed. Practical steps include identifying the important parameters to be analyzed and working backwards through the decision tree to identify how to obtain a suitable sample for the specific technology and parameter of interest. Following this process, a sampling protocol and sample log is constructed. Technological constraints sometimes present significant challenges for sampling, however, in most cases, reliable samples can be obtained once a thorough analysis of the process plan is conducted.

9.8 *Composting Technology Systems and Sample Collection*—The physical/mechanical nature of the feedstock preparation and composting operation may impose constraints on sampling. Each composting technology imposes specific limitations on sampling. Representative samples may not be obtainable with some technologies. Therefore, a facility's sampling plan must take into account the realistic strategy for obtaining representative samples. In general, highly engineered compost processes impose more constraints on sampling than a simple composting process. For example, outdoor windrows are more easily sampled than large rotating drums.

9.8.1 Ten basic types of composting systems are presented in Fig 02.01-A3 and their associated sampling constraints are outlined in Table 02.01-A1. Each system introduces particular traits or constraints that impact how (and why) samples are collected. New forms of compost technology under development may expand the list, but the generic form of the prescribed models cover most existing composting technologies.

9.8.2 *Sampling Plan Basics*—The two process-focused modes of compost sampling are: i) In-Process sampling for monitoring during a specific composting technology process; and ii) End-Process sampling. There may be multiple steps or multiple processes involved in an overall system. Sample collection for testing commonly occurs at the end of a specific step of the composting process, mostly for convenience and to be certain that the sample is representative of the batch. Sample collection during a process imposes significant constraints because of the inherent variability of in-process materials. Sampling at these points must be

carefully designed to sample across any existing gradient of non-uniformity.

9.8.3 Discussion in the following section identifies technologies and primary constraints or requirements for representative sampling.

9.8.3.1 *Type A. Home Bins* come in many shapes and sizes, from fixed solid containers, loose wooden structures to rotating solid-tanks. The appropriate framework for sampling is to select the material representing the finished product. Some systems provide doors at the bottom of a bin from which samples may be easily removed; other bins require disassembling or removal from the pile and hand-mixing of the mass. Precaution must be taken to assure a homogenous mixture under any circumstance.

NOTE 7—The inclusion of home composting bins in TMECC is not a suggestion or endorsement for regulatory control, but for information and perspective only. While home composting bins are not a mainstay of commercial composting and not currently or likely to be regulated by state or local jurisdictions when the end product is used by the home generator and producer, the principles described in TMECC for assessing overall quality of compost are suitable for use on such products.

9.8.3.2 *Type B. Turned Windrows* are either batch or continuous piles. In the former common case, the entire windrow is made from similar ingredients at about the same time (e.g., within 3 d). In the latter case, materials are added lengthwise over time. In both cases, non-uniformity is observed down the length of the pile and is greatest with continuous modes of composting. Sampling of windrows requires compositing over a discrete length, either the entire pile, or a sub-section identified to have similar age or other characteristics. Windrow turning machines are useful for preparing uniform mixtures suitable for composite sampling; however, a single pass with a turning machine will not result in an evenly mixed pile, 3-4 passes commonly are required. If turning is performed frequently, the need for multiple turns prior to sampling diminishes.

9.8.3.3 *Type C. Static Piles* are recognized for their non-uniformity. These piles exhibit gradients of temperature, aeration and exposure to elements that reduce homogeneity over time. To obtain a representative sample from a static pile, extreme disruption and mixing is required. Breaking down the pile with a bucket loader and re-mixing after removal of the outer cover may be necessary. If mixing is not complete, sub-samples should be taken from each region during pile breakdown, or from the bucket as material is removed. However, if the purpose of sampling is to characterize non-uniformity, then effort must be made to get to the region of concern where a representative sample can be collected. This could be performed using a core sampler, or by breaking open the pile with heavy equipment.

9.8.3.4 *Type D. Agitated-Bed* systems generally move compost along the length of the system at a fixed rate per day. Should sampling be necessary during the process, care must be taken to understand the variability imposed by nature of daily additions to the system. In some cases, the actual technology physically restricts access for various reasons including worker safety. In such situations, samples can be collected at the discharge end where material comes off the bin. Several sub-samples should be taken each day, cooled immediately; and several days' accumulated samples (except for bacteriological and others parameters limited by a 48 h holding time) can be composited to form a bulk sample.

9.8.3.5 *Type E. Enclosed Vessel* reactors are either circular or oblong containers, bins or towers (these systems may or may not contain internal moving parts) and cannot be easily accessed for sampling. Sample collection is best performed at the vessel's discharge end. In-process sampling for quality control and process monitoring is not always practical with these systems.

9.8.3.6 *Type F. Rotating Vessels* are horizontal tanks, usually positioned on a gradient. They are used for continuous and sometimes for batch composting. Most systems do not have ports to access the material during processing, making in-process sampling impractical. As with the enclosed vessel design, sampling is usually performed at the discharge end of the vessel. Rotating vessels are often used during "Feedstock Preparation" for many technology types, and sampling is performed on the download conveyor.

9.8.3.7 *Type G. Cure Piles* are frequently very large and may contain material composited from several piles. Because of their heterogeneity and size, and the typical lack of turning and mixing, they usually display extreme gradients of moisture, maturity and bulk density. Under these circumstances, one effective way to adequately sample is to use a large tractor loader to break into the pile, moving and mixing the materials in the process. The sampling plan must incorporate a stratified sampling scheme and point sampling to distinguish gradients and map spatial non-uniformity.

9.8.3.8 *Type H. Bagged Product* results from a mixing and screening process that is assumed to produce uniform material prior to bagging. Additional mixing of the bulk mass after bagging and prior to sampling is precluded. Therefore, a statistically representative sample must consist of many sub-samples collected from different bags. Additionally, the physical constraint of extracting small sample cores from separate bags that are palletized compounds the problems of collecting proper samples.

9.8.3.9 *Type I. Source Ingredients* are notorious for non-uniformity. Large sub-samples that accurately

represent the distribution of ingredients must be well mixed, and if possible (when appropriate), shredded to reduce the sample size while retaining sample integrity. Large mechanical equipment may improve the sample collection and preparation process.

9.8.3.10 *Type J. Lab Systems* are a special case of composting and are usually handled as a discrete sampling problem on an individual institutional basis. However, with the increasing popularity of bench scale testing, particularly for bioremediation composting, the value of describing sample units and types becomes more obvious. In general, these units contain highly uniform materials and are sometimes so small that the entire unit becomes the sample from which sub-samples are drawn for separate analyses. Because non-uniformity increases with miniaturization, lab systems are usually designed with small openings into discrete sections of tanks to facilitate extraction of small sub-samples. This allows the operator to monitor the formation of gradients and non-uniformity in miniature lab systems.

Table 02.01-A1 Sampling operations, constraints and required tools for ten types of composting technologies.

Type	*Sampling Action*	*Constraints*	*Preferred Tools*
A. Home Bins	Must open bin, remove cover and sides, and mix by hand	Not homogenous, may be hard or impossible to open	Pail and spading fork
B. Turned Windrows	Sample after turning with machine from surface of pile if well mixed	Pile varies along length, turning machine may not homogenize in one pass	5-gal pail, spading shovel, corer
C. Static Piles	Remove chip cover, and dig into depth, may require bucket loader and multiple depth sampling	Extreme non-uniformity, layering and clumping, inadvertent mixing with cover or surface residues; may be sealed inside tube	5-gal pail, spading shovel, corer or auger, bucket loader
D. Agitated-Bed	Sample after turning or agitation event, or sample discharge	Difficult access except at discharge, piles vary along length with age of source	5-gal pail, spading shovel,
E. Enclosed Vessel	Sample from side doors or top port after agitation	Very difficult or impossible access; potential layering	5-gal pail, spading shovel, corer, auger
F. Rotating Vessels	Sample from discharge/output end or take-away conveyor	Difficult or impossible to sample except at discharge; output varies with time	5-gal pail, shovel or scoop
G. Compost Curing Piles	Remove chip cover, and dig into depth, may require bucket loader and multiple depth sampling	Very large piles, non-uniformity, difficult access, compaction and layering; surface cover mixing	5-gal pail, spading shovel, corer, auger, bucket loader
H. Bagged Product	Sample multiple bags, cores drawn	Bag damage, difficult access	5-gal pail, trowel or soil-corer
I. Source Ingredients	Composite from each pile separately, remove surface	Non-uniformity may be great, poorly mixed, difficult access	Large pail, shovel; bucket loader
J. Lab Systems	Open system and remove with core sampler	Small scale, difficult access	5-gal pail, Spatula, trowel, soil-corer

9.9 *Sampling Interval*—There are no process-specific formulas that dictate sampling intervals for source ingredients and compost, except when biosolids are composted (Table 02.01-A2). Sampling intervals of composting materials for reporting purposes may be fixed by certain regulations. It is advisable to consult local or state sampling guidelines. As a general rule, incoming feedstocks should be sampled every two weeks, or every 3,000 to 5,000 tons of finished product.

9.9.1 *Formula to estimate sampling interval, d:*

$$S = T \div F \times R \qquad \textit{Equation 9.9.1}$$

where:

S = sampling interval in days, d
T = sampling threshold in tons (e.g., 4,000 t), t,
F = tons of incoming feedstock per day, t d^{-1}, and
R = weight reduction factor of incoming feedstock, %.

9.9.2 *Weight Reduction Factor, R:*

$$R = C \div F \qquad \textit{Equation 9.9.2}$$

where:

R = weight reduction factor of incoming feedstock, %,
C = mass of finished compost per week, t dw, and
F = mass of incoming feedstock per week, t dw

NOTE 1A—If the actual weight reduction factor is unknown, use 0.70 until the actual value can be determined. Refer to Method 03.09 Total Solids and Moisture for a description of how to determine dry weight of compost and feedstocks.

Table 02.01-A2 Sampling intervals for composted biosolids.

Amount produced (metric tons of biosolids compost per 365-day period)	*Monitoring Frequency for Pathogens and Trace Elements*
< 290	Once per year (1 yr^{-1})
≥ 290 to < 1,500	One per quarter (4 times yr^{-1})
≥ 1,500 to < 15,000	Once per 60 days (6 times yr^{-1})
≥ 15,000	Once per month (12 times yr^{-1})

Adapted from US EPA 40CFR503

9.9.3 *Sampling raw source ingredients—Example 1.* Samples shall be taken from incoming material that has been shredded, tumbled or otherwise reduced in particle size. From the material exiting the shredder/mixer, one point-sample shall be obtained every 2 h, over an operational period of 6-8 h, for a total of 4 samples. Sample size should be approximately 1000 cm^3 (~ 1 qt) per sample. The four samples shall then be thoroughly mixed together (composite), and a portion of the mixture (composite sub-sample) taken for analysis. If point-sampling directly from the shredder or mixing mill is not possible, the incoming material shall be sampled no more than 24 h after passing through the shredding equipment.

9.9.4 *Example 2—Sampling compost materials.* For each sampling event, a single composite sample shall be made up of multiple sub-samples for each pile or batch, unless otherwise directed.

9.9.5 *Example 3—Sample locations.* Construct and label a diagram of sample locations for your composting system. The example provided in TMECC 02.01-B indicates a minimum of fifteen sub-samples per pile. This procedure establishes a composite or general characterization of the attributes in a compost pile.

9.9.5.1 Refer to section 02.01-B for a strategy to sample generic windrows of compost.

9.9.5.2 Samples collected during the composting process are not composited in the same manner as finished samples because point-specific problems must be identified and monitored. Factors such as anaerobic materials and volatile fatty acids (VFA) may need to be determined from point-samples extracted from multiple locations in the same pile.

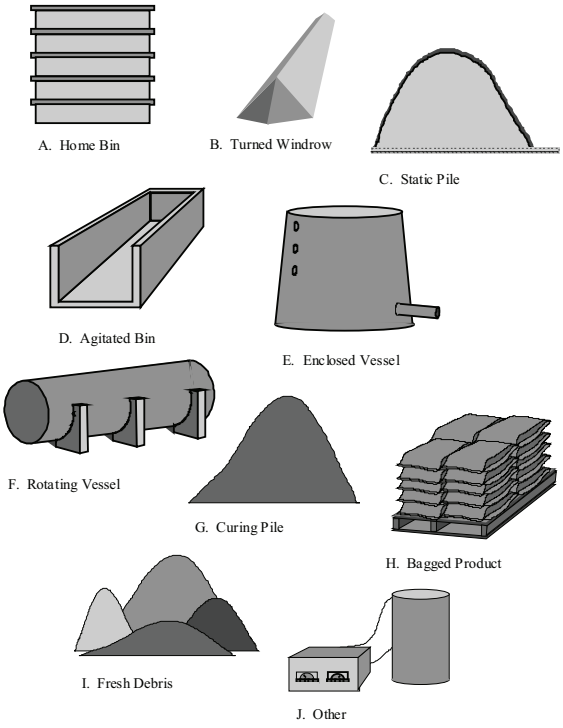


Fig 02.01-A3 Composting technologies.

9.9.6 *Example 4—Sample Variance Exercise.* The coefficient of variation (CV) expresses the relative variability for a parameter of interest across multiple samples. The CV is expressed as a percentage and calculated by dividing the sample standard deviation by the sample mean and multiplied by 100.

9.9.6.1 The ability to distinguish differences between arithmetically similar sample values decreases as the CV increases. It is difficult to draw specific conclusions about analytical results when variability is high. Under circumstances where variability is consistently high either the sampling plan must be redesigned to account for the excessively high variability, or the parameter should be discarded as a standard measure.

9.9.6.2 Consider a hypothetical case where two standard parameters are used to evaluate compost stability, C:N and VFA. Assume that the upper limit of acceptable variability for the parameters are set at 15% for C:N, and 45% for VFA. Low CV thresholds are generally assigned to system and process critical measures, and high CV thresholds are assigned to less critical standard measures.

NOTE 2A—This is a hypothetical case. It may be very difficult to establish meaningful CV limits without a large amount of data from many composts across time for a given test parameter. In addition, depending on the test, an individual test parameter may show a very large CV for repeated analysis of one sample.

9.9.6.3 In the example given in Table 02.01-A2, the CV for VFA testing is greater than the CV for C:N analysis, but the latter is unacceptable, given the use of the data, whereas the former is acceptable. In this hypothetical case, large variations across VFA samples are less significant than smaller variations associated with C:N. This is because variations in VFA's are transient and either readily corrected or do not diminish compost quality relative to its intended use, whereas highly variable C:N ratios indicate potentially serious problems with the composting process and product quality.

Table 02.01-A3 Compost sample data analyzed for variability

Sample	*C:N Ratio*	*VFA mg kg^{-1}*
1	35	12,000
2	19	18,000
3	39	19,000
4	22	25,000
5	42	9,000
Average:	31.4	16,600
Standard Deviation:	10.3	6,268
%CV:	33	38
Acceptable CV:	15%	45%
Suitability of Data:	REJECT	ACCEPT

9.10 *Sampler Devices*—There is no single standardized compost sampling device. Tools and devices for soil and forage sampling are relatively simple and efficient and are useful for compost sampling, but they have severe limitations.

9.10.1 *Slotted Tube Sampler*—Single or double, slotted tube and rod, all slotted ends and a minimum 5-cm (2-in.) diameter. The Pennsylvania State Forage Sampler, or equivalent, is a satisfactory core sampler for composts that do not contain significant foreign objects.

9.10.2 *Shovel*—Standard long, handled, pointed tip; typical horticultural narrow shovel, cleaned well with soapy water, rinsed, and dried between samples.

9.10.3 *Thief Sampler,*

9.10.4 *Trier,*

9.10.5 *Pipe*—PVC or plastic,

9.10.6 *Tarpaulin*—plastic,

9.10.7 *Pail*—16- to 20-L (4- to 5-gal), square pails. Use standard 5-gal plastic pails only when square pails are not available (e.g., square pails are available through Cleveland Bottle & Supply Co.; 850 East 77th Street; Cleveland, OH 44103; telephone: 216 881 3330; FAX: 216 881 7325; URL: http://www.clevelandbottle.com/squrpail.html). Pails must be cleaned well with soapy water, rinsed, and dried between samples.

9.10.8 *Trowel*—Standard garden, high-density polypropylene (HDPP) for sub-sample mixing and bag-filling; trowels must be cleaned well with soapy water, rinsed, and dried between samples.

9.10.9 *Sample Containers*—Use a container that is appropriate for the laboratory analysis to be performed on the collected compost sample. Refer to Tables 02.01-2 through 02.01-6, and Figure 02.01-B3.

9.10.10 Labels and Logbook

9.10.10.1 *Labels*—Name of technician, operator, inspector, facility name, pile identification, date, time, sample number and location in pile using length, width and height coordinates from an identified end and depth from surface measured perpendicular to surface, purpose of sample/test, method of sample preservation.

9.10.10.2 *Logbook*—Name of technician, operator, or inspector; and facility name. Pile data including: pile identification; feedstock-mix; type of pile; date started; weather conditions at time of sampling (for exposed piles only); pile orientation relative to natural drainage. Sample data including: date and time of sample collection; location where samples were collected in pile using length, width and height coordinates from an identified end and depth from surface measured horizontally; description of the sampling point; purpose of sample/test, method of sample preservation, point or composite sample; number and volume of the samples taken; date and time samples were shipped.

Test Method: Selection of Sampling Locations for Windrows and Piles						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		02.01-B	02.01-B	02.01-B	02.01-B	02.01-B	02.01-B	02.01-B

02.01-B SELECTION OF SAMPLING LOCATIONS FOR WINDROWS AND PILES

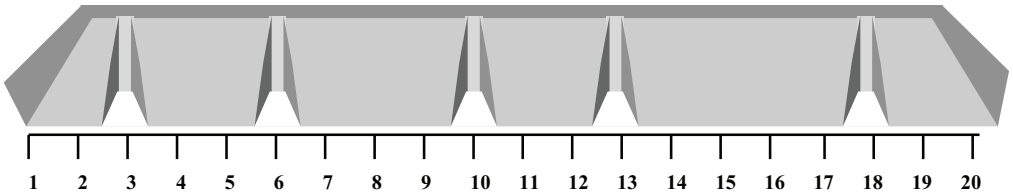


Fig 02.01-B1 Hypothetical sample collection pattern from a compost windrow.

NOTE 1B—In this example, a scale from 1-20 is superimposed on the long dimension of a compost windrow. Five distances (3, 6, 10, 13 and 18 m) are randomly selected to each side of the windrow, (e.g., numbers randomly pulled from a hat), to assign sample collection locations. Point-samples are collected from within three zones at each cutout.

NOTE 2B—The illustrated cut-outs are depicted on one side of the windrow; in a real operation, the cut-outs must be randomly assigned to each side of the windrow. Cone-shaped piles have a circular base. Measure around the base of a cone-shaped pile and randomly assign cutout positions along the pile's meridian, or circumference.

10. Apparatus for Method B

10.1 *Sampling Container*—five 16- to 20-L (4- to 5-gal), plastic (HDPP), glass.

10.1.1 *Organic Contaminant Tests*—For samples to be analyzed for the presence of organic contaminants, please refer to Table 02.01-6 Organic Contaminant Tests: Sampling containers and conditions for compost and source ingredient testing. Modify sample packaging steps presented in this section accordingly.

10.2 *Sampling Device*—silage auger, tilling spade, or other appropriate sampling device.

10.3 *Tractor Loader*—with loader, (e.g., Bobcat, etc.).

10.4 *Trowel*—high-density polypropylene (HDPP), for stirring and mixing composite sample.

10.5 *Pail*—16- to 20-L (4- to 5-gal), square pails, Use standard 5-gal plastic pails for shipping only when square pails are not available (e.g., square pails are available through Cleveland Bottle & Supply Co.; 850 East 77th Street; Cleveland, OH 44103; telephone: 216 881 3330; Fax: 216 881 7325; URL: http://www.clevelandbottle.com/squrpail.html).

11. Reagents and Materials for Method B

11.1 *Plastic Bags*—three 4-L (1 gal) durable bags with seal, (e.g., Ziploc® **Freezer** bags).

11.2 *Plastic Gloves.*

11.3 *Tarp*—clean plastic, canvas, or other type of mixing surface if feedstock is liquid sludge.

11.4 *Cold Packs*—chemical ice packs, or 4-L plastic bags (e.g., heavy duty Ziploc® freezer bags) filled with approximately 0.5 L of water and frozen flat. One ice pack per 4-L sample container of compost to be shipped, (e.g., three ice packs are recommended for three compost 4-L samples).

11.5 *Aluminum Foil*—lining for plastic shipping pail, and

11.6 *Packing Material*—newspaper or other appropriate bulking material to be used as packing or fill to minimize sample movement within the shipping container (square pail) during shipping.

11.7 *Adhesive Tape*—duct tape, 5-cm (2-in.) width.

12. Procedures for Method B

12.1 *Cut into Finished Compost*—Using tractor skid-loader, bobcat or shovel, or sample boring device, cut into the finished compost pile or windrow at five or more randomly selected positions. Collect samples from the full profile and breadth of the compost windrow or pile. Refer to Fig 02.01-B1.

12.2 *Collect Point-Samples*—Samples of equal volume are extracted from the compost pile at three depths or zones measured from the pile's uppermost surface. Collect no less than five point-samples from each of the three depths or zones illustrated in Fig 02.01-B2. The five point samples for each zone must be collected in a manner to accurately represent the horizontal cross-section of the windrow or pile. Use a sanitized sampling tool (a gloved hand, clean shovel or auger) when collecting samples and when transferring samples to the 5-gal sample collection pail.

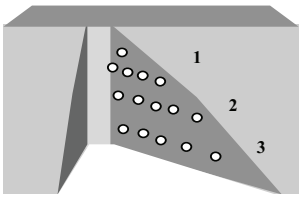


Fig 02.01-B2 Five horizontally dispersed point-samples are collected from each of three depths or zones within each cutout.

NOTE 3B—(1) upper $^1/_3$ of compost profile height; (2) middle $^1/_3$ of compost profile height; and (3) lower $^1/_3$ of compost profile height, where compost pile does not exceed the recommended overall height of 3 m. Create more than three sampling depths or zones within each cutout when the curing pile exceeds a height of 3 m, relative variability is high or the property of interest is found at very low concentrations, near the laboratory detection limit.

12.3 *Composite Point-Samples*—Place all 15 point samples from one cutout together into one sanitized plastic pail. Completely mix the point samples by stirring thoroughly with a sanitized wooden stick or lath, and by covering and shaking the pail to further mix the samples.

12.3.1 Repeat the blending process at least four times until all point samples are thoroughly blended to form one composite sample that accurately represents the compost for the cutout.

12.3.2 Proceed to the next compost sample cutout and repeat this process to collect one thoroughly blended composite sample from each of the five cutouts.

12.3.3 *Composite Sample*—Transfer the five composite samples from the sample collection pails onto a mixing tarp or other appropriately sanitized surface or container, such as into a large pail where all samples can be mixed, blended and then covered to minimize moisture loss. Thoroughly blend the five composite samples to form one large sample that represents the average condition of the entire batch or windrow in question.

12.3.3.1 Quarter the composite sample and thoroughly mix and quarter again. Continue to subdivide and split the sample into quarters and mix as described until sample size reaches approximately 12 L (3 gal).

12.4 *Stratified Sampling*—This sample collection strategy is used to evaluate for the presence of spatial variations or gradients in compost characteristics across and through a windrow or pile.

12.4.1 *Stratified Samples across Cutouts*—Use this sampling strategy to test for differences in compost characteristics between sample cutouts and along the longer dimension of a windrow. Do not composite materials from the five separate cutouts when monitoring for the presence of gradients along the longer dimension of a windrow. Pack and prepare five separate samples (i.e., five separate composite samples, one from each cutout) for shipment as described in step 12.5.

12.4.2 *Stratified Samples within Cutouts*—Use this sampling strategy to evaluate for the presence of spatial variations or gradients that occur with changes in pile depth or distance from the windrow core to its surface.

12.5 Prepare for Shipment and Storage:

12.5.1 Transfer the blended compost to three 4-L (1-gal) sample bags, (e.g., plastic Ziploc® *freezer* bags).

12.5.2 Line the shipment pail with aluminum foil or other reflective material to minimize sample heat-gain. Place the sample bags containing the compost sample into the plastic pail and interleave with ice packs for shipping (refer to Fig 02.01-B3).

12.5.3 Cover the pail with its lid. Seal and secure the lid with a packing tape. Send the sample pail by one-day express delivery service to your selected laboratory for analysis. Include a chain of custody information sheet with environmental regulatory samples (Refer to Method 02.01-E).

NOTE 3B—Maintain cool samples at 4°C (39.2°F) to diminish microbial and chemical activity prior to and during sample shipment.

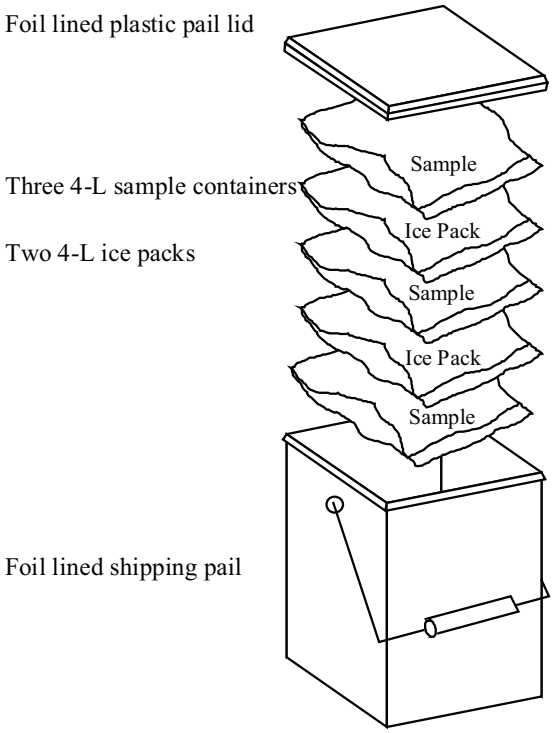


Fig 02.01-B3 Preparation for shipment.

Test Method: Field Sampling Plan for Composted Material						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
02.01-C	02.01-C	02.01-C	02.01-C	02.01-C	02.01-C	02.01-C	02.01-C	02.01-C

02.01-C FIELD SAMPLING PLAN FOR COMPOSTED MATERIAL

13. US EPA SW-846 Guideline Review and Considerations

13.1 With its hazardous waste management system, the US EPA requires that certain solid wastes be analyzed for physical and chemical properties. In its hazardous waste management system, it is mostly chemical properties that are of concern, and in the case of a number of chemical components, the US EPA has promulgated levels (regulatory thresholds) that cannot be equaled or exceeded.

13.1.1 Regulations pertaining to the management of hazardous waste contain three references regarding the sampling of solid wastes for analytical purposes:

13.1.1.1 Collect representative samples of waste, so that they exhibit average properties of the bulk compost or feedstock.

13.1.1.2 Collect enough samples (but no less than four samples) over a period of time sufficient to represent the variability of the compost or feedstock.

13.2 *Sampling Plan Implementation*—The US EPA manual contains a section on implementation of the sampling plan (SW-846 Chapter Nine, part 2). Within that section there is discussion concerning the sampling program's objectives for evaluating a compost. (Refer to Fig 03.01 Sample fate).

13.2.1 The example suggests the following questions be answered:

13.2.1.1 Is the sampling being performed to comply with environmental regulation?

13.2.1.2 Samples are to be analyzed for which parameters?

13.2.1.3 Why not others?

13.2.1.4 Should samples be analyzed for fewer parameters?

13.2.1.5 What is the end-use of the generated data?

13.2.1.6 What are the required degrees of accuracy and precision?

13.2.2 These questions may or may not be as important for sampling composted solid waste.

13.3 *Sampling Plan Considerations*—The implementation section contains a category entitled Sampling Plan Considerations. The sampling plan is usually a written document that describes the objectives, and details the individual tasks and how they will be performed. The more detailed the sampling plan, the less opportunity for oversight or misunderstanding during sampling, analysis, and data management.

13.3.1 The SW-846 document suggests that a sampling plan be designed with input from the various sectors involved in the project, including the following personnel:

13.3.1.1 *regulatory sampling*—in many cases may require state permits and consultations with state officials.

13.3.1.2 *end-user*—to use the data to attain program objectives.

13.3.1.3 *field team member*—an experienced member of the field team who actually collects samples.

13.3.1.4 *analytical chemist*—to review analytical requirements for sampling, preservation, and holding times that will be included in the sampling plan.

13.3.1.5 *process engineer or equivalent*—it explain details and constraints of the production process being sampled.

13.3.1.6 *statistician*—to review the sampling approach and verify that the resulting data will be suitable for any required statistical calculations for decisions.

13.3.2 *quality assurance representative*—to review the applicability of standard operating procedures and determine the number of blanks, duplicates, spike samples, and other steps required to document the accuracy and precision of the resulting data.

13.3.3 If no one is familiar with the site to be sampled, then a pre-sampling site visit should be arranged to acquire site-specific information. Some modifications of the sampling plan may be necessary. It is necessary to have at least one experienced sampler as a member of a sampling team.

14. Statistical Validity of Sampling Plan

14.1 *Objectives*—The primary objective of a sampling plan for a compost is to collect an appropriate

number of representative samples and subsamples for accurate and precise measurement of the chemical, physical and biological properties of the compost. If the chemical measurements are sufficiently accurate and precise, they will be considered reliable estimates of the chemical properties of the compost.

14.1.1 Generally, high degrees of accuracy and precision are required if one or more chemical components of compost are present at a concentration that is close to the applicable regulatory threshold. Alternatively, relatively low accuracy and low precision can be tolerated if the components of concern occur at levels far below or far above their applicable thresholds. Low sampling precision is often associated with considerable savings in analytical costs, as well as expenses associated with sampling; and is clearly recognizable even in the simplest of statistical tests. However, low sampling accuracy may not entail cost savings and is always obscured in statistical tests (i.e., it cannot be evaluated). Although it is often desirable to design sampling plans for compost to achieve only the minimally required precision (at least two samples are required for any estimate of precision), it is prudent to design the plans to attain the greatest possible accuracy.

14.2 *Composite Sampling*—For composite sampling, a number of random subsamples are initially collected and combined into a single sample, which is analyzed for the chemical constituents of concern. The major disadvantage of composite sampling, as compared with non-composite sampling, is loss of information about the spatial variability of chemical constituents because only a single estimate of the parameter is generated. The benefit is that a credible, general representation of the entire compost pile is generated from a large number of subsamples which are composited.

14.3 *Sampling Quality Assurance/Quality Control (QA/QC):*

14.3.1 Make sure all sampling equipment and containers are clean. If equipment is used to collect multiple samples, provisions for cleaning and decontamination are required between samples.

14.3.2 Properly label all samples and keep accurate records. Record as much information on sample labels as possible prior to arriving at the site. Sample labels and field notes should include material type, location, date, approximate age of compost, sampler's name, special sampling procedures used, analytical procedures to be performed, preservatives added (if any), and any special observations or incidents during the sampling event.

14.3.3 Point-samples must be stored in a refrigerator (4°C) before analysis when delays in shipment to laboratory are anticipated. This preservation is especially important for feed stock samples, compost to be evaluated for stabilization or maturity, or microbiological analysis. Chemical quality changes that may take place due to microbiological activity between sample collection and laboratory analysis should be avoided.

14.3.4 Chain of custody forms and procedures should be used with all environmental samples.

14.4 *Other Sampling Considerations*—Compost samples are taken at each facility for a variety of purposes. Varying levels of expertise and quality assurance are required depending on the sampling purpose or objective. A unique sampling protocol should be developed for each specific objective. This information should be detailed in a facility operation and maintenance (O&M) manual and be accessible to all facility staff.

14.4.1 Key process variables including porosity, nutrient balance, oxygen, moisture, temperature and time are monitored and controlled on a continual or daily basis. Measurements of weight and volume of waste arriving and compost leaving the facility are necessary for planning material movements, personnel and transportation requirements, and maintaining facility aesthetics. Although this is the most frequent type of sampling conducted, the sampling quality assurance requirements are the least significant for these activities. Generally, process control and material handling data do not need to be precise to be useful, (e.g., appropriate application of quick-tests). Regulatory compliance and product attribute data must be highly precise and accurate, (e.g., statistically valid sampling program to accurately estimate the average value of interest).

14.5 *Sampling Frequency*—Operating permits for compost sites require that concentrations of certain constituents of environmental concern be evaluated, (e.g., As, Ba, Cd, Cu, Cr, Hg, Mn, Mo, Ni, Pb, Se, Zn, pathogens such as *Salmonella* and fecal coliform, and organic compounds such as PCB's, PCP's, dioxins, furans, organochlorine and organophosphorus pesticides). Regulatory agencies establish compliance using individual sample results. It is, therefore, very important that sample collection and preparation techniques provide representative samples.

NOTE 1C—As much as 20,000 m^3 of compost may be represented by one subsample as small as 1 g. Because of this, it is vital that the sample be representative of the total material. *Quality control and quality assurance for quarterly testing must be greater than that employed for routine daily monitoring.*

14.6 *Statistical Techniques*—Statistical techniques for obtaining accurate and precise samples are relatively simple and easy to implement. Accurate representations of an entire compost pile or batch may be achieved through random sampling. In random sampling, every unit in the population has a theoretically equal chance of being sampled and

measured. Consequently, statistics generated by the sample (e.g. sample mean and to a lesser degree, standard deviation) are unbiased estimators of true population parameters. That is, the sample is representative of the population. A common method of selecting a random sample is to divide the population by an imaginary grid, assign a series of consecutive numbers to the units of the grid, and select the number to be sampled using a random-numbers table.

NOTE 2C—Haphazardly selected samples are not random and therefore not a suitable substitute for a randomly selected sample. That is because there is no assurance that a person performing undisciplined sampling will not consciously or subconsciously favor the selection of certain units of the population.

14.6.1 Sampling precision is achieved by collecting the appropriate number of samples that are uniformly distributed across the entire volume of compost. Precision is improved by increasing the number of samples, while maintaining a sampling pattern to guarantee a spatially uniform distribution.

14.6.2 If a batch of compost is randomly heterogeneous with regard to its chemical characteristics and if that random chemical heterogeneity remains constant from batch to batch, accuracy and appropriate precision can usually be achieved by simple or systematic random sampling. More complex stratified random sampling is appropriate if a batch of compost is known to be non-randomly heterogeneous in terms of its chemical properties and non-random chemical heterogeneity is known to exist from batch to batch. In such cases, the population is stratified to isolate the known sources of non-random chemical heterogeneity. The units in each stratum are numerically identified, and a simple random sample is taken from each stratum. This type of sampling would be advantageous only if the stratification efficiently divides the waste into strata that exhibit maximum between-strata variability and minimum within-strata variability. In composted solid waste that is frequently turned and mixed, little if any stratification is likely to occur. If little or no information is available concerning the distribution of chemical components, simple or systematic random sampling are the most appropriate sampling strategies.

14.7 *Number of Samples*—The appropriate number of samples to collect is the least number required to generate a sufficiently precise estimate of the true mean concentration of a chemical component of a compost. From the compost producer's perspective, this means that the minimum number of samples needed to demonstrate that the upper limit of the confidence interval for the true mean is less than the applicable regulatory threshold value. It is always prudent to collect a greater number of samples than indicated by preliminary estimates of the mean and variance since poor preliminary estimates of those statistics can result in an underestimate of the appropriate number of samples to collect.

14.8 *Simple Random Sampling*—For convenience, the statistical calculations for simple random sampling (wherein within-batch heterogeneity that may be encountered by a compost producer is low) are provided (adapted from SW-846 Chapter Nine, part 2, pages 13-14).

14.8.1 Obtain preliminary estimate of $\bar{x}$ for each chemical component of compost that is of concern. The above-identified statistic is calculated by Equation 14.8.1.

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} \qquad \textit{Equation 14.8.1}$$

where:

$\bar{x}$ = simple random sample mean,
n = total number of sample measurements,
x = variable in question (e.g., mercury),
i = individual samples ranging from 1 to n, and
$\sum_{i=1}^{n} x_i$ = sum of all x's (analytical results for individual samples), from i = 1 through i = n.

14.8.2 Obtain preliminary estimate of variance, s^2, for each chemical component of concern. The above-identified statistic is calculated by Equation 14.8.2.

$$s^2 = \frac{\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2 \Big/ n}{n-1} \qquad \textit{Equation 14.8.2}$$

where:

s^2 = variance of simple random sample,
n = total number of sample measurements,
x = variable in question (e.g., mercury), and
i = individual samples ranging from 1 to n.

14.8.3 Estimate the appropriate number of samples (n_1) to be collected from the compost through use of Equation 14.8.3 and Table 02.01-C1. Derive individual values of n_1 for each chemical component of concern (x). The appropriate number of samples to be taken from the compost is the greatest of the individual n_1 values.

$$n = \frac{t_{.20}^2 s^2}{\Delta^2} \qquad \textit{Equation 14.8.3}$$

where:

n = number of samples,
$t_{.20}^2$ = tabulated "t" value for two-tailed confidence interval and a probability of 0.20,
s^2 = sample variance, and

Δ^2 = the square of the regulatory threshold minus sample average, defined by US EPA, (e.g., 100 mg kg^{-1} for barium in elutriate of EP toxicity).

Table 02.01-C1 Tabulated values of Student's "t" for evaluating compost.

Degrees of freedom (n-1)	*Tabulated "t" value*	*Degrees of freedom (n-1)*	*Tabulated "t" value*
1	3.078	16	1.337
2	1.886	17	1.333
3	1.638	18	1.330
4	1.533	19	1.328
5	1.476	20	1.325
6	1.440	21	1.323
7	1.415	22	1.321
8	1.397	23	1.319
9	1.393	24	1.318
10	1.372	25	1.316
11	1.363	26	1.315
12	1.356	27	1.314
13	1.350	28	1.313
14	1.345	29	1.311
15	1.341	30	1.310
		40	1.303
		60	1.296
		120	1.289

14.8.3.1 Randomly collect at least n_1 (or $n_2 - n_1$, $n_3 - n_2$, etc., as will be indicated in step 8) samples from the compost. Maximize the physical size (volume) of all samples that are collected from the strata.

NOTE 3C—Collection of a few extra samples will provide protection against poor preliminary estimates of $\bar{x}$ and s^2.

14.8.3.2 Analyze the n_1 (or $n_2 - n_1$, $n_3 - n_2$, etc.) samples for each chemical component of concern. Superficially (graphically) examine each set of analytical data from each stratum for obvious departures from normality.

14.8.4 Calculate the standard deviation *(s)* for each set of analytical data by Equations 14.8.1, 14.8.2, 14.8.4 and 14.8.5.

$$s = \sqrt{s^2} \qquad \textit{Equation 14.8.4}$$

14.8.5 Calculate $\bar{x}$, s^2, and standard error (s_x) for each set of analytical data by, Equations 14.8.1, 14.8.2, and 14.8.5.

$$s_x = \frac{s}{\sqrt{n}} \qquad \textit{Equation 14.8.5}$$

14.8.5.1 If $\bar{x}$ for a chemical component is equal to or greater than the applicable regulatory threshold (from Equation 14.8.3) and is believed to be an accurate estimator of μ (population mean), the component is considered to be present in the compost at a hazardous concentration, and the study is completed. Otherwise, continue the study. In the case of a set of analytical data that does not exhibit obvious abnormality and for which $\bar{x}$ is greater than s^2, perform the following calculations with non-transformed data. Otherwise, consider transforming the data by the square root transformation (if $\bar{x}$ is about equal to s^2) or the arcsine transformation (if $\bar{x}$ is less than s^2) and performing all subsequent calculations with transformed data.

14.8.6 Determine the confidence interval (CI) for each chemical component of concern by Equation 14.8.6. If the upper limit of the CI is less than the applicable regulatory threshold (applied in Equation 14.8.3), the chemical component is not considered to be present in the compost at a hazardous concentration, and the study is completed. Otherwise, the opposite conclusion is tentatively reached.

$$\text{CI} = \bar{x} \pm t_{0.20} s_x \qquad \textit{Equation 14.8.6}$$

where:

$t_{0.20}$ = referred to in Table 02.01-C1 Tabulated values of Student's "t" for evaluating compost for appropriate degrees of freedom.

14.8.7 If a tentative conclusion of hazard is reached, re-estimate the total number of samples *(n_2)* to be collected from the compost by use of Equation 14.8.3. When deriving n_2, employ the newly calculated (not preliminary) values of $\bar{x}$ and s^2. If additional $n_2 - n_1$ samples of compost cannot reasonably be collected, the study is completed, and a definitive conclusion of hazard is reached. Otherwise, collect an extra $n_2 - n_1$ samples of compost.

14.8.8 Repeat the basic operations described in Steps 14.8.3 through 14.8.7 until the compost is judged to be non-hazardous or, if the opposite conclusion continues to be reached, until increased sampling effort is impractical.

14.9 *Stratified Random Sampling*—For convenience, the statistical calculation steps for stratified random sampling that must be performed in situations that may be encountered by a compost producer where within-batch heterogeneity is high are provided below (from SW-846 Chapter Nine, part 2, pages 18-19).

14.9.1 Obtain preliminary estimate of $\bar{x}$ for each chemical component of concern. The identified statistic is calculated by Equation 14.9.1.

$$\bar{x} = \sum_{k=1}^{r} W_k \bar{x}_k \qquad \textit{Equation 14.9.1}$$

where:

$\bar{x}$ = stratified random sample mean,

$\bar{x}_k$ = stratum mean, and

W_k = fraction of population represented by stratum *k* (number of strata *[k]* range from 1 to *r*).

14.9.2 Obtain preliminary estimate of s^2 for each chemical component of compost that is of concern. The identified statistic is calculated by Equation 14.9.2.

$$s^2 = \sum_{k=1}^{r} W_k s_k^2 \qquad \textit{Equation 14.9.2}$$

where:

s^2 = stratified random sample variance,

s_k^2 = stratum variance, and

W_k = fraction of population represented by stratum *k* (number of strata *[k]* range from 1 to *r*).

14.9.3 Estimate the appropriate number of samples (n_1) to be collected from the compost through use of Equation 14.8.3 and Table 02.01-A1 Tabulated values of Student's "t" for evaluating compost. Derive individual values of n_1 for each chemical component of concern. The appropriate number of samples to be taken from the compost is the greatest of the individual n_1 values.

14.9.4 Randomly collect at least n_1 (or $n_2 - n_1$, $n_3 - n_2$, etc., as will be indicated in step 8) samples from the compost. If s_k for each stratum (see Equation 14.9.2) is believed to be an accurate estimate, optimally allocate samples among strata (i.e., locate samples among strata so that the number of samples collected from each stratum is directly proportional to the s_k for that stratum). Otherwise, proportionally allocate samples among strata according to size of the strata. Maximize the physical size (volume) of all samples that are collected from the strata.

14.9.5 Analyze the n_1 (or $n_2 - n_1$, $n_3 - n_2$, etc.) samples for each chemical component of concern. Superficially (graphically) examine each set of analytical data from each stratum for obvious departures from normality.

14.9.6 Calculate $\bar{x}$, s^2, the standard deviation *(s)*, and $s_{\bar{x}}$ for each set of analytical data by, respectively, Equations 14.9.1, 14.9.2, 14.8.4 and 14.8.5.

14.9.7 If $\bar{x}$ for a chemical component is equal to or greater than the applicable regulatory threshold (from Equation 14.8.3) and is believed to be an accurate estimator of μ (population mean), the component is considered to be present in the compost at a hazardous concentration, and the study is completed. Otherwise, continue the study. In the case of a set of analytical data that does not exhibit obvious abnormality and for which $\bar{x}$ is greater than s^2, perform the following calculations with non-transformed data. Otherwise, consider transforming the data by the square root transformation (if $\bar{x}$ is about equal to s^2) or the arcsine transformation (if $\bar{x}$ is less than s^2) and performing all subsequent calculations with transformed data.

14.9.8 Determine the confidence interval (CI) for each chemical component of concern by Equation 14.8.6. If the upper limit of the CI is less than the applicable regulatory threshold (applied in Equation 14.8.3), the chemical component is not considered to be present in the compost at a hazardous concentration, and the study is completed. Otherwise, the opposite conclusion is tentatively reached.

14.9.9 If a tentative conclusion of hazard is reached, re-estimate the total number of samples (n_2) to be collected from the compost by use of Equation 14.8.3. When deriving n_2, employ the newly calculated (not preliminary) values of $\bar{x}$ and s^2. If additional $n_2 - n_1$ samples of compost cannot reasonably be collected, the study is completed, and a definitive conclusion of hazard is reached. Otherwise, collect an extra $n_2 - n_1$ samples of compost.

14.9.10 Repeat the basic operations described in Steps 14.9.3 through 14.9.9 of Fig 02.01-1 Composting Unit Operations, until the compost is judged to be non-hazardous or if the opposite conclusion continues to be reached until increased sampling effort is impractical.

Test Method: Composting Feedstock Material Sampling Strategies						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
02.01-D	02.01-D							

02.01-D BATCH FEEDSTOCK MATERIAL SAMPLING STRATEGIES

15. Apparatus for Method D

15.1 *Sampling Container*—20-L (5-gal), stainless steel, plastic, glass or Teflon.

15.2 *Sampling Device*—wooden spatula or tiling spade, etc.

15.3 *Trowel*—high-density polypropylene (HDPP).

15.4 *Plastic Storage Pail*—20-L (5-gal), square pails, Use standard 5-gallon plastic pails only when square pails are not available (e.g., Cleveland Bottle & Supply Co.; 850 East 77th Street; Cleveland, OH 44103; telephone: 216 881 3330; Fax: 216 881 7325; URL: www.clevelandbottle.com/squrpail.html.

16. Reagents and Materials for Method D

16.1 *Plastic Gloves*.

16.2 *Tarp*—clean plastic, canvas, or other type of mixing surface if feedstock is liquid sludge.

16.3 *Plastic Bags*—three 4-L (1 gal) Ziploc® *freezer* bags.

16.4 *Cold Packs*—chemical ice packs,

16.5 *Aluminum Foil*—lining for plastic shipping pail, and

16.6 *Adhesive Tape*—duct tape, 5-cm (2-in.) width.

17. Procedure for Method D

17.1 *Sample Collection*—Identify and collect an appropriate number of subsamples needed to ensure a reliable analytical result as described in Methods 02.01-A, B or C.

17.1.1 Place each subsample into a sampling (subsample) container.

17.1.2 Transfer the contents of the subsample container onto (into) mixing surface (container) and proceed to the next randomly selected sample point.

17.1.3 Repeat steps 17.1.1 and 17.1.2 until the predetermined number of subsamples is obtained.

17.2 *Sample Mixing*—Place subsamples on clean tarp or other similar mixing platform, mix sub-samples thoroughly using a wooden spatula or comparable sampling tool.

17.3 *Sample Splitting*—Subdivide sample into quarters, thoroughly mixed composite sample into fourths. Repeat steps 17.2 and 17.3 until sample size is appropriate for intended analysis.

17.4 *Sample Storage and Shipping*—Place composite sample aliquot in clean container, preferably a Teflon pail or similar inert material.

CAUTION—Do not use galvanized sheet metal collection or mixing tools. The galvanized coating will contaminate the sample with zinc.

17.4.1 Transfer blended feedstock or compost to fill three 4-L (1-gal) plastic Ziploc® freezer bags.

17.4.2 Line the shipment pail with aluminum foil to minimize heat exchange. Place the plastic Ziploc® freezer bags containing the feedstock samples in the plastic pail and interleave with cold packs for shipping (refer to Fig 02.01-B3).

17.4.3 Seal the square pail with its lid. Seal and secure lid with duct tape. Send the square plastic pail containing samples by two-day express service to the selected laboratory for analysis. Include completed chain of custody forms when necessary.

NOTE 1D—If any delay is anticipated, cool sample to 4°C (39.2°F) to diminish microbial and chemical activity prior to sample shipment.

Test Method: Data Quality Management and Sample Chain of Custody						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
02.01-E	02.01-E		02.01-E			02.01-E	02.01-E	02.01-E

02.01-E DATA QUALITY MANAGEMENT AND SAMPLE CHAIN OF CUSTODY

18. Aspects of Sampling Quality Assurance for Reported Data

18.1 Three critical steps in the sampling process precede laboratory analysis and often dictate data quality.

18.1.1 sample planning and collection;

18.1.2 sample handling and preservation; and

18.1.3 laboratory sample preparation.

18.2 Each step in the sampling process must be properly executed in a timely manner by well informed, trained individuals to ensure that the collected sample accurately represents a compost batch, windrow or pile.

18.3 *Quality Sample Management*—Regulatory and certification systems may dictate that samples are properly collected, preserved and prepared for analysis. Consider the following hypothetical example of sample management where a certified third party is introduced to manage the sampling plan.

18.3.1 The third party assumes all quality assurance and quality control responsibilities associated with:

18.3.1.1 sample planning and collection;

18.3.1.2 sample handling and preservation; and

18.3.1.3 laboratory sample preparation.

18.3.2 Responsibility for rigorous sample collection is transferred from facility management to the third party. Responsibilities associated with sample storage, preparation and laboratory analysis are also transferred from the analytical laboratory to the third party.

18.3.3 One of the principal benefits of the third party sampling system is to diminish deviations in sampling plan interpretation and implementation across separate facilities and laboratories. Third party control can decrease variability by maintaining consistent field sampling protocols across all participating facilities. Field sample collections would be implemented as described in *TMECC 02.01 Field Sampling of Compost Materials*. Consistent sample preparation protocols would also be followed for laboratory analysis as described in *TMECC 02.02 Laboratory Sample Preparation for Analysis*.

18.4 *Tracking Quality*—A sample must be properly collected and prepared for shipment, and then properly manipulated by laboratory personnel who follow specific preparation protocols designed for each analytical methodology. Previous sections emphasized the importance of properly designed and implemented sampling plans. This section introduces a protocol designed to modify data interpretation to interpret sample variability.

18.4.1 Consider the following hypothetical sampling plan that incorporates an additional step to verify accuracy of reported results using cross-validation techniques. One type of a statistically valid sample management plan requires that samples are properly collected at a very high frequency while the actual number of samples submitted for analysis remains small.

18.4.1.1 *Establish Baseline*—A significant number of samples that represent the composting process of a facility are collected over time and sent to a laboratory for analysis. Results from these samples serve to establish a baseline of information that accurately represents the compost produced by the facility and a given feedstock blend.

18.4.1.2 *Track Deviations from Baseline*—After the baseline is established, samples are collected at specified intervals, over time or per unit of compost produced (refer to *TMECC 02.01-A Equation 9.9.1 Formula to estimate sampling interval*), and held in cold storage. After a specified interval, (e.g., quarterly or monthly) a small but statically representative number of prepared samples are randomly selected from the stored samples and sent to a laboratory for analysis. Because multiple samples would be randomly selected from a larger population of samples, a more reliable statistical inference can be generated than by simply directly submitting monthly or quarterly samples for analysis.

18.4.2 Sampling programs of this nature may require that field samples, or samples prepared for laboratory analysis, are submitted to a secure or bonded cold-storage facility where frequently collected samples are inventoried and properly stored. Samples must be retained in storage for a predetermined time period to

safeguard against cases where a need for re-testing may arise.

18.4.3 *Sampling Costs*—Sampling program maintenance costs should be considered when designing an effective monitoring system. It is difficult to weigh the relative importance of data quality when there is no clear relationship between financial outcome and monitoring protocol. Successful implementation will increase when data quality relates to an increased financial incentive, either artificially through incentives offered by the governing regulatory agency or through quality assurance certification programs designed to indirectly increase market share.

02.01 SUMMARY

19. Report

19.1 Chain of custody forms and procedures should be used with all environmental or regulatory samples. A chain of custody form is used to track sample handling from time of collection through laboratory analysis, and data reporting. Suggested information for the chain-of-custody record includes, at a minimum: Collector's name; Signature of collector; Date and time of collection; Place and address of collection; Requested preprocessing (subsampling, compositing, sieving); Requested analyses; Sample code number for each sample (if used); Signature of the persons involved in the chain of possession. Refer to Fig 02.01-E1 Chain of Custody form for an example.

20. Keywords

20.1 accuracy; aliquot; attribute verification; bias; chain of custody; closed vessel system; composite; compost; coefficient of variation; %CV, confidence interval; feedstock; grab-sample; point-sample; point-sampling; open vessel system; precision; process monitoring; process variability; product variability; quality control; quality assurance; representative sample; sample collection frequency; sampling; sampling plan; statistical validity; stratified sampling; windrow.

STA Laboratory: _____ *Tel:* _____
Address: _____ *FAX:* _____
_____ *Email:* _____
City, State Zip code: _____

Client/Reporting Company: _____ *Tel:* _____
Contact Name: _____ *FAX:* _____
Billing Address: _____ *Email:* _____
_____
City, State Zip code: _____

Send Results to: _____
City, State Zip code: _____

Name or Source of sample(s): _____
Name of Sample Collector: _____

LABORATORY USE ONLY *Storage Locations*

Freezer _____ Cold Room _____ Storage Shelf _____

_____ _____ _____ _____ _____ _____ _____ _____ _____

Sample Condition:

Sample Type: O COMPOSITE O POINT O STRATIFIED

P.O. Number: _____
Client STA ID: _____
USCC Member: O YES O NO ID: _____

SELECTION OF ANALYSIS. *Refer to http://tmecc.org/sta for details.*
STA Suite; All 503 Rule Tests; Other – Specify additional tests in fields A through D (below).

NOTE ! *Your selection of **STA Suite** (below) authorizes laboratory personnel to disclose all analytical results AND submit the STA Compost Technical Data Sheet directly to STA program management.*

A	B	C	D

	Client Sample Identification (and special instructions)	*Collection Date/Time*	*Sample Matrix*	*Sample Container*	*Shipping Temp.*	*Selected Analysis* STA	503	A	B	C	D	*Lab/Job Number*
1		*Date:*	Compost O	Plastic Bag O	Ambient O	O	O	O	O	O	O	1
		Time:	Feedstock O	Pail O	Wet Ice O							
		Initials:	_____ O	_____ O	Dry Ice O							
2		*Date:*	Compost O	Plastic Bag O	Ambient O	O	O	O	O	O	O	2
		Time:	Feedstock O	Pail O	Wet Ice O							
		Initials:	_____ O	_____ O	Dry Ice O							
3		*Date:*	Compost O	Plastic Bag O	Ambient O	O	O	O	O	O	O	3
		Time:	Feedstock O	Pail O	Wet Ice O							
		Initials:	_____ O	_____ O	Dry Ice O							
4		*Date:*	Compost O	Plastic Bag O	Ambient O	O	O	O	O	O	O	4
		Time:	Feedstock O	Pail O	Wet Ice O							
		Initials:	_____ O	_____ O	Dry Ice O							
5		*Date:*	Compost O	Plastic Bag O	Ambient O	O	O	O	O	O	O	5
		Time:	Feedstock O	Pail O	Wet Ice O							
		Initials:	_____ O	_____ O	Dry Ice O							

Releasing Signature	Date	Time	Receiving Signature	Date	Time
1			1		
2			2		
3			3		
4			4		

Fig 02.01-E1 Chain of Custody. Compost sample chain of custody log

Test Method: Laboratory Sample Preparation. Six Protocols						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		02.02-A		02.02-A		02.02-A		02.02-A
			02.02-B	02.02-B	02.02-B	02.02-B		02.02-B
				02.02-C	02.02-C	02.02-C	02.02-C	02.02-C
				02.02-D	02.02-D	02.02-D		02.02-D
				02.02-E	02.02-E	02.02-E		02.02-E
	02.02-F	02.02-F						

02.02 LABORATORY SAMPLE PREPARATION

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers laboratory sample handling and preparation for physical, chemical and biological laboratory analysis.

1.1.1 *Method 02.02-A Sample Mixing and Splitting.*

1.1.2 *Method 02.02-B Sample Sieving for Aggregate Size Classification.*

1.1.3 *Method 02.02-C Man Made Inert Removal and Classification.*

1.1.4 *Method 02.02-D Milling and Grinding Samples, Harrison.*

1.1.5 *Method 02.02-E Milling and Grinding Samples, Munter.*

1.1.6 *Method 02.02-F Modifications for Feedstock Sample Preparation.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

1.3 *The methodologies described in this section do not purport to address all safety concerns, if any, associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to their use.*

1.4 *This document is a working draft and is not approved for publication.* All methods and sampling protocols provided in TMECC are subject to revision and update to accommodate new widely accepted advances in techniques and methods.

1.5 The process alternatives, trade names, or commercial products are only examples and are not endorsed or recommended by the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1.6 Omissions and errors should be reported to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

2. Referenced Documents

2.1 *TMECC:*

Method 03.05-A Film Plastic Surface Area Determinations Using Digital Processing.

Method 03.06-A Glass Shards, Metal Fragments and Hard Plastics Wet Sieving Technique.

2.2 *Other References:*

ASTM D 2977-71, Standard Test Method for Particle Size Range of Peat Materials for Horticultural Purposes. *In* Annual Book of ASTM Standards, Vol. 04.08.

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3. Terminology

3.1 *aliquot, n*—a subsample of a material prepared for, and subjected to laboratory analysis. A subsample size smaller than 1 g may be used to represent more than 1000 kg of compost.

3.2 *air-dry weight, n*—Weight of sample material air-dried in a forced air oven at 36°C for 48-72 h.

3.3 *as-received weight, n*—Mass of a sample with a moisture content of that at time of receipt by a laboratory.

3.4 *fresh weight, n*—Weight of undried sample material, stored and maintained at the same moisture content as at time of receipt.

3.5 *oven dry weight (ODW), n*—Weight of sample material dried in a forced air oven at 70±5°C for 18-24 h, or until sample weight change diminishes to nil.

3.6 *working sample, n*—Sieved (square mesh) fresh material. Less than 9.5 mm fraction.

4. Summary of Test Methods

4.1 *Method 02.02-A Sample Mixing and Splitting*—The bulk sample is thoroughly blended. The blended sample is split into separate components using a stainless steel sample splitter. One portion (4L, 1 gal) of the sample is placed in frozen storage (-4°C) as backup, and the remaining portions are placed in cold storage (4°C) or further processed for laboratory analysis.

4.2 *Method 02.02-B Sample Sieving for Aggregate Size Classification*—A 4-L aliquot of as-received material is passed through a series of nested sieves. The moisture and total solids content are determined for each size fraction and the sieve size distribution of the bulk sample is calculated.

4.3 *Method 02.02-C Man Made Inert Removal and Classification*—Perform this test on size classed samples (aliquot size up to 250 cm^3). Inerts are hand sorted and classified for each size fraction. After the inerts > 4 mm, and sharps > 2 mm are removed from this fraction, the total fraction < 9.5 mm is milled for the metal and LOI OM analysis, and other tests that call for where air-dried, milled samples.

4.3.1 Inert removal prior to milling and acid digestion decreases the incidence of inflated metal analysis. The air-dried *working sample* aliquot with inerts removed is milled to a powder and stored in a sealed plastic bottle to minimize accumulation of hygroscopic moisture. Tests performed on milled air-dried (36°C) samples are heavy metals, plant nutrients (except N), and organic carbon (OC).

4.4 *Method 02.02-D Milling and Grinding Samples, Harrison*—A large sample (250 cm^3) of relatively coarse, oven-dried material is milled with a Wiley Mill into a fine dust and thoroughly blended. Small aliquots (< 5 g) of the milled material are selected to represent the bulk sample for elemental analysis.

4.5 *Method 02.02-E Milling and Grinding Samples, Munter*—Preferred method to minimize sample heterogeneity. A large sample (250 cm^3) of relatively coarse, air-dried material (< 9.5 mm) with inerts removed is milled with a Stein Mill equipped with a carbide blade into a fine dust and thoroughly blended. Small aliquots (< 5 g) of the milled material are selected to represent the bulk sample for elemental analysis.

4.6 *Method 02.02-F Modifications for Feedstock Sample Preparation*—A large sample (1000 cm^3) of relatively coarse material is milled into a fine dust and thoroughly blended. Small aliquots (< 5 g) of the milled material are selected to represent the bulk sample for elemental analysis.

CAUTION—To avoid metal contamination, heavy metals analyses should be performed only on materials that are milled with carbide-tipped blades.

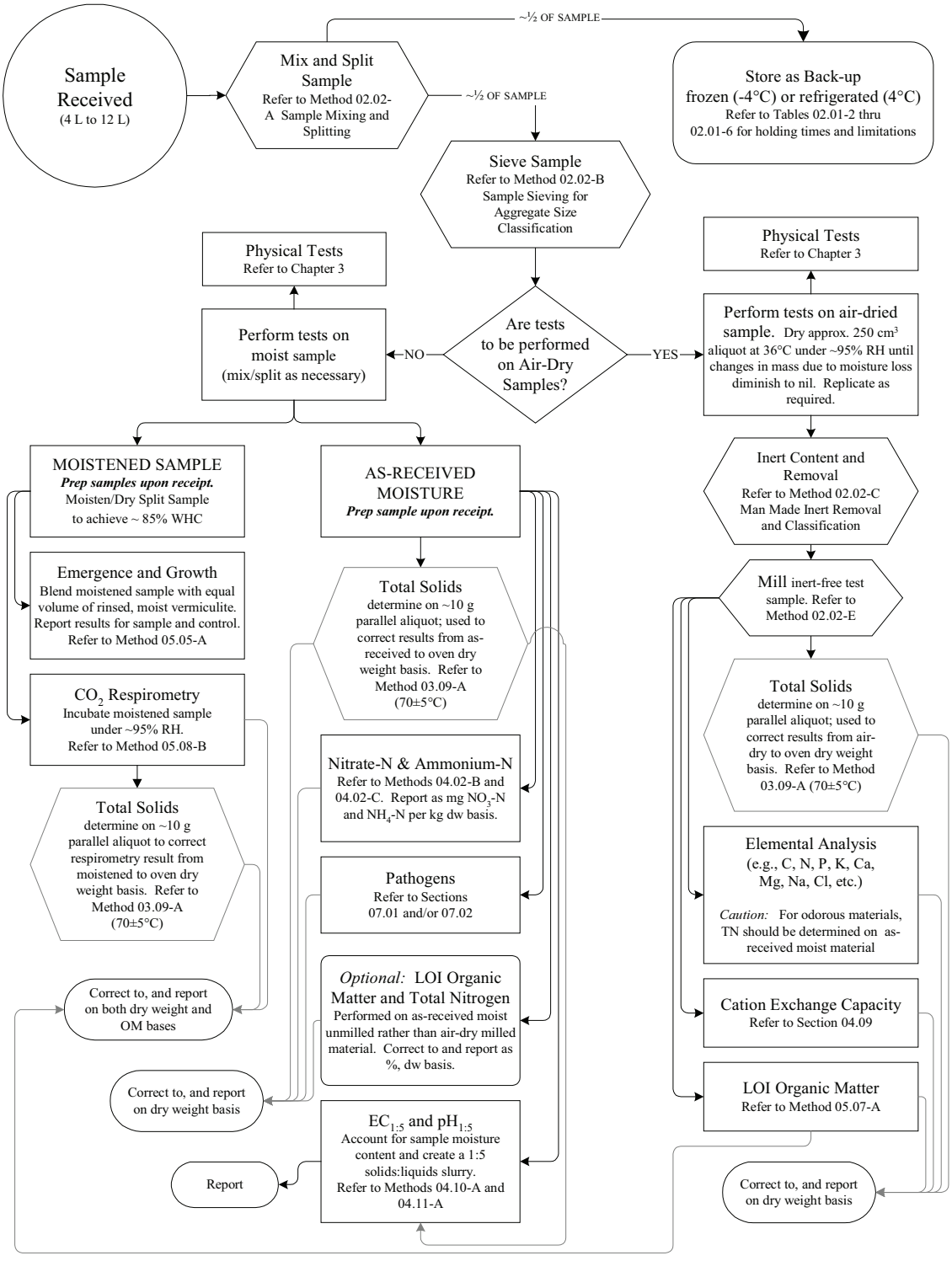

Fig 02.02-1 Flow of sample fate through laboratory sample preparation. Only common routine testing is included in this illustration. Please note that a majority of physical tests (TMECC Chapter 03) are performed on as-received moist material. Notable exceptions include one of the water-holding capacity tests and both wettability tests which call for dried materials. Additional tests performed on as-received moist material not listed in the diagram include all other respirometry methods, bioassays, VFA's, other pathogens and synthetic organic compounds. Refer to specific TMECC sections and methods for detailed descriptions of, or deviations in, sample preparation protocols.

5. Significance and Use

5.1 *Method 02.02-A Sample Mixing and Splitting*—Sample heterogeneity is a serious limiting factor for biological, chemical and physical analysis. Poor analytical precision is often related to inadequate sample handling, preparation and blending. For example, compost subsamples may arrive at the laboratory in three separate bags instead of as three bags of a composite sample. A composite sample is blended and subdivided in the field at the time of sampling.

5.2 *Method 02.02-B Sample Sieving for Aggregate Size Classification*—Sample heterogeneity can be attributed to variations in physical, biological and chemical characteristics across and within sub-samples. Sample variability is often stratified across aggregate sizes. Segregation of a sample according to aggregate size fractions facilitates differentiation of these factors across material size fractions and their corresponding degrees of degradation and other forms of heterogeneity.

5.2.1 Tests performed on moist samples include sieve size classification, total N, NH_4^+, NO_3, pH, electrical conductivity, respirometry, pathogen testing, germination and growth, bulk density, water holding capacity (WHC), porosity/pore space, volatile fatty acids (VFA) and LOI organic matter.

5.2.2 Tests performed on samples air-dried at 36°C include inert count, and after milling include heavy metals, plant nutrients (including total N for mature material low in ammonia), and organic carbon (OC).

5.2.3 Tests performed on samples oven dried at 70±5°C include total solids and moisture, and volatile solids.

5.3 *Method 02.02-C Man Made Inert Removal and Classification*—Inert materials include metals, hard and film plastics, glass shards, sharps (sewing needles, straight pins and hypodermic needles), and large stones and wood fragments. Inert removal prior to acid digestion decreases the incidence of inflated metal analysis.

5.4 *Method 02.02-D Milling and Grinding Samples, Harrison*—A milled sample can be well mixed to create a relatively homogeneous material. From this milled material, small aliquots of 1 to 2 g can be analyzed for metals and other constituents with high precision relative to the entire milled sample.

5.5 *Method 02.02-E Milling and Grinding Samples, Munter*—Sample homogeneity is increased by thoroughly blending and milling a sample. The technique improves precision of elemental and chemical analyses by homogenizing a sample aliquot (250-cm^3) that represents the bulk sample.

5.6 *Method 02.02-F Modifications for Feedstock Sample Preparation*—Refer to specific test methods for applications.

6. Interference and Limitations

6.1 Standardized sample preparation will minimize error in analytical results.

6.2 Samples must be thoroughly mixed and blended before extracting an aliquot for analysis. Recall, as much as 20,000 m^3 of compost is represented by one sample aliquot as small as 0.5 g. It is vital that the received sample be thoroughly blended. Quality control and quality assurance must be employed for all compost materials.

6.2.1 Excessive sample handling can increase evaporative and volatile loss of some compounds of interest. Loss is diminished when samples are handled and prepared in a cold-room at approximately 4°C.

6.2.2 *Micro-nutrients and heavy metals analysis*—Sample containers must be pre-washed with detergents, (e.g., 10% RBS, P-free and B-free), acids, and Type II water. Plastic and glass containers are suitable. Refer to Chapter 3 in Annual Book of ASTM Standards for detailed information.

6.3 *Method 02.02-B Sample Sieving for Aggregate Size Classification*—Most sample analyses are performed on material sieve fractions smaller than 9.5 mm. Larger fractions are generally evaluated for their inert content and relative contribution to the volume and mass of the bulk sample, and as bulking materials used to manage pile porosity. Material smaller than 2 mm is soil by definition, and is considered too small to evaluate for inert content using standard dry sieving methods.

6.4 *Method 02.02-C Man Made Inert Removal and Classification*—The < 9.5 mm material is dried at a lower temperature (36°C) to minimize loss of volatile compounds and elements such as mercury.

6.4.1 This method for removal of inerts is performed on air-dried (36°C) sample material. Inert materials can easily be over-looked, especially when many samples are processed too rapidly. The process is time consuming and should not be rushed.

6.5 *Method 02.02-D Milling and Grinding Samples, Harrison*—This method was devised for compost samples that do not contain foreign materials such as glass, metal or plastics. This method is not recommended for MSW or yard waste composts that may contain man-made inerts.

6.6 *Method 02.02-E Milling and Grinding Samples, Munter*—Grinding pure quartz sand erodes the metal blade by a minor amount relative to the amount of metals found in compost. The < 2-mm sieve fraction of compost may contain abrasive sand, glass and metal

that will erode the carbide blade, but not to the degree found after milling pure quartz sand.

6.7 *Method 02.02-F Modifications for Feedstock Sample Preparation*—Refer to specific methods for details.

7. Sample Handling

7.1 *Finished Compost*—Approximately 12 L (3 gal) of compost material are needed to complete a full suite of analytical procedures including physical, biological and chemical tests. The entire composite sample should be blended and split upon receipt. If any delay is anticipated, the material should be placed in cold storage (4°C) until sample preparation can continue. The sample must be retained for no more than 14 d in cold storage. Material must be blended in a closed container to minimize evaporative water loss.

7.2 *Samples for Pathogen Analysis*—Sterile whirl packs are often used for samples to be analyzed for pathogen content. A separate whirl pack should be used for each analytic sample, i.e., prepare one aliquot for each sample. Samples should be analyzed immediately or stored appropriately for the type of pathogen assays to be conducted (Refer to Table 07.00-2).

7.3 *Preservation and Storage of Samples:*

7.3.1 *Short-Term Storage < 24 h*—Moist material should be placed in cold storage (4°C) until sample preparation is initiated to minimize microbial and chemical activity that could alter the material's characteristics. Air-dried milled material and oven-dried material should be stored in sealed containers such as plastic or glass bottles at room temperature.

7.3.2 *Long-Term Storage > 24 h*—Moist material should be placed in frozen storage (-4°C) until sample preparation is initiated to stop microbial and chemical activity. Air-dried, milled material and oven-dried material should be placed in cold storage (4°C) in sealed containers such as plastic or glass bottles after no more than 14 d to minimize microbial and chemical activity. Air-dried milled material and oven dried material should be stored frozen (-4°C) in sealed containers such as plastic or glass bottles for periods in excess of 14 d.

Test Method: Laboratory Sample Preparation. Sample Mixing and Splitting						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		02.02-A			02.02-A	02.02-A		02.02-A

02.02-A SAMPLE MIXING AND SPLITTING

CAUTION—Always clean and disinfect equipment before processing a second sample.

NOTE 1A—This method is based upon procedures developed for municipal solid waste (MSW) compost materials at the University of Minnesota Research Analytical Lab , Department of Soil, Water and Climate, St. Paul.

8. Apparatus for Method A

8.1 *Twin Shell Dry Blender*—tumbling mixer modified to hold a closed 20 L (5-gal) plastic pail, (e.g., LB-2191 - The Patterson-Kelly Co. Inc. East Stroudsburg, PA).

8.2 *Sample-Splitter, and Divider*—stainless steel with 2 in. wide chutes, stainless steel collection pans, (e.g., Soil Test Model Cl-286, Lake Bluff, IL. 60014).

9. Reagents and Materials for Method A

9.1 *Water*—deionized (DI), 17 MΩ·cm or purer.

9.2 *Storage Bags*—4-L (1-gal) plastic Ziploc®.

9.3 *Whirl Packs*—200-mL (e.g., Nasco whirl-pak®).

10. Procedure for Method A

10.1 *Sample Mixing*—Tumble as-received (fresh) sample with mixer in sealed 20-L plastic pail for <30 min to assure complete blending. This allows mixing without artificial drying.

10.2 *Sample Splitting*—Split the sample by passing it through a stainless steel splitter/divider; use stainless steel or plastic collection pans.

10.3 *Sample Storage*—Store approximately one-third to one-half as reserve in a freezer (-4°C). The remaining sample portion (4 L to 8 L) is sieved as outlined under Sample Sieving for the Particle Size and other tests.

10.4 *Whirl Pack Storage*—Store at 4°C up to a maximum of 30 h.

CAUTION !—*Excessively Moist Samples* need to be partially air-dried prior to mixing, to minimize the formation of balls and clumps. Spread excessively moist material on a large plastic bag, place in open air, intermittently mixing with gloved hand until the proper moisture level is reached (approximately 40-50% moisture). Check the sample every 6-12 h; do not over-dry.

CAUTION !—Do not use galvanized sheet metal sieves, collection pans or mixing pails. The galvanized coating will contaminate the sample with zinc.

Test Method: Laboratory Sample Preparation. Sample Sieving for Aggregate Size Classification							**Units:** *NA*	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
			02.02-B	02.02-B	02.02-B	02.02-B		02.02-B

02.02-B SAMPLE SIEVING FOR AGGREGATE SIZE CLASSIFICATION

COMMENT—After splitting the mixed sample, the larger subsample is immediately weighed and passed through nested sieves (e.g., 50-mm, 25-mm, 16-mm, 9.5-mm, 6.4-mm, and 4-mm) for the aggregate size classification.

CAUTION !—Excessively moist compost, >85% WHC, (Refer to Method 03.10), will clump and compact during the sieving process.

11. Apparatus for Method B

11.1 *Sieves*—20-cm (8-in.) diameter, stainless steel frame and cloth: 50-mm (2-in.), 25-mm (1-in.), 16-mm (⅝-in.), 9.5-mm (⅜-in.), 6.4-mm (¼-in., optional), and 4-mm (⅛ in., #5), (e.g., Gilson Company Inc., Worthington, OH 43085-0677, or equivalent).

11.2 *Mechanical Shaker*—for nested sieves, (e.g., Tyler Model RX-86, or equivalent).

11.3 *Beakers*—100-mL, 250-mL.

11.4 *Drying Oven*—forced air.

12. Reagents and Materials for Method B

12.1 *Drying Containers*—capable of retaining mass at temperatures above 75°C, (e.g., paper bags, brown, #4).

13. Procedure for Method B

13.1 *Weigh Sample Aliquot*—Weigh and record the fresh weight of at least 2 L (½ gal), but no more than 6 L (1½ gal) of the received compost, assuming total sample size of 4 L (1 gal).

NOTE 2B—An equal volume shall be placed in long term frozen storage (-4°C) - Refer to Method 02.02-A Sample Mixing and Splitting.

13.1.1 If delays are anticipated, the *working sample* scheduled for further analysis should be placed in cold storage (4˚C) from which sub samples are removed as required for different tests.

13.2 *Nested Sieving:*

13.2.1 Nest the sieves and transfer approximately 250 cm^3 aliquot of bulk material onto the top sieve (50 mm); cover and secure the nested sieves onto the mechanical shaker set for 5 min.

13.2.2 Place the respective size fractions into corresponding labeled and tared drying containers (e.g., #4 paper bags or other suitable containers).

13.2.3 Repeat these steps until the entire sample is sieved and sorted according to size fraction.

13.3 *Sample Splitting*—Material that passes through the 9.5-mm sieve is subdivided into three separate aliquots.

13.3.1 A small aliquot (30-50 cm^3) is oven dried at 70±5°C for 24 to 36 h in 100-mL beaker to determine wet basis sample moisture.

13.3.2 A larger aliquot of each fraction (250-300 cm^3) is air-dried at 36°C for approximately 48-72 h.

13.3.3 The remainder is placed in cold storage (4°C) for tests performed on moist sample material.

13.4 *Determine Moisture Content*—Each of the remaining size fractions is placed in tared drying containers, weighed and oven dried at 70±5°C for 24-72 h (until sample weight change diminishes to nil) to determine moisture content.

CAUTION—Do not flash dry in a microwave. Flash drying elevates temperature above 36°C and can create small spheres of charred material around metal filings.

13.5 *Reporting Basis*—The results for all tests are reported on a weight basis adjusted to a 70±5˚C dry weight (both moist and air-dried aliquots).

COMMENT—Tests are performed on both moist samples and air-dried samples. It is important to follow the instructions given for each specific test. Samples targeted for metals analysis are to be air-dried under forced air at 36˚C for approximately 48-72 h.

14. Calculations for Method B

14.1 *Moisture Content (wet basis; %)*—correct all weights to 70±5°C dw basis.

14.1.1 *Air-Dried Samples (36°C):*

$$M = [1 - (A \div F) \times (O \div A)] \times 100 \qquad \textit{Equation 14.1.1}$$

14.1.2 *Oven-Dried samples (70±5°C):*

$$M = (1 - O \div F) \times 100 \qquad \textit{Equation 14.1.2}$$

where:

A = air dry weight at 36°C,

O = oven dry weight at 70±5°C, and

F = weight of material at as-received moisture.

14.2 *Determine Sieve Size Distribution:*

14.2.1 *For each sieve fraction, calculate:*

$$R_i = O_i \div O_B \times 100 \qquad \textit{Equation 14.2.1}$$

where:

R_i = relative contribution of sieve size fraction "i" to bulk weight of sample, %,

O_i = oven dry weight of individual sieve size fractions (dried at 70±5°C), g,

i = sieve size fractions of interest, (e.g., < 4-mm, 4-mm to 6.4-mm, 6.4-mm to 9.5-mm, 9.5-mm to 16-mm, 16-mm to 25-mm, 25-mm to 50-mm, and >50-mm), and

O_B = oven dry weight of bulk sample (dried at 70±5°C), before sieving, g.

Test Method: Laboratory Sample Preparation. Man Made Inert Removal and Classification							**Units:** *NA*	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
				02.02-C	02.02-C	02.02-C	02.02-C	02.02-C

02.02-C MAN-MADE INERT REMOVAL AND CLASSIFICATION

NOTE 1C—This method is based upon procedures developed for MSW compost materials at the University of Minnesota Research Analytical Lab., Department of Soil, Water and Climate, St. Paul, MN 55108.

COMMENT—This test was designed to be performed in conjunction with Method 02.02-B Sample Sieving for Aggregate Size Classification.

15. Apparatus for Method C

15.1 *Tweezer.*

15.2 *Analytical Balance*—1 mg to 1 kg.

15.3 *Oven*—forced air drying set at 70±5˚C.

15.4 *Weighing Trays*—four (one for each inert class and stones).

15.5 *Sieves*—2-mm, 4-mm, and 9.5-mm.

15.6 *Lab Tray*—45 x 65 cm (18 x 26 in.) pressed fiberglass, or other smooth surfaced material.

15.7 *Mechanical Shaker*—for nested sieves, (e.g., Tyler Model RX-86).

16. Reagents and Materials for Method C

16.1 *Sample Containers*—capable of retaining mass at temperatures near 75˚C, (e.g., 0.5-L rigid plastic storage containers).

17. Procedure for Method C

17.1 Sieve an adequate volume of as-received moist bulk sample using the 9.5-mm sieve to generate a 250 cm^3 sample aliquot of < 9.5-mm material.

17.1.1 Transfer approximately 250 cm^3 of the material which passes the 9.5-mm sieve to a separate clean container (minimum sample size of 250 cm^3). Obtain and record the gross fresh weight, (±0.001 g).

17.1.2 Set aside materials that do not pass through the 9.5-mm sieve for later processing (step 17.5).

17.1.3 Air-dry the < 9.5-mm material in a vented oven set at 36˚C. Continue to dry the sample until weight loss diminishes to nil, (e.g., for approximately 36 h to 48 h). Obtain and record the gross air-dry weight of the 250-cm^3 test aliquot.

17.2 *Separate sample by size fraction:*

17.2.1 Sieve the 250-cm^3 test aliquot of the air-dried material using a 4-mm sieve. Transfer material that passes the 4-mm sieve to a separate container. Obtain and record the mass of the < 4-mm fraction and set aside for further processing (step 17.4).

17.2.2 Transfer the remaining fraction (> 4-mm) to a clean lab tray or other flat, smooth surface.

17.3 *Inerts between 4-mm and 9.5-mm*—Remove, separate, and classify all pieces of plastic, metal, glass and stones. Obtain and record the mass of inert class.

NOTE 2C—Stone removal will minimize damage to the *Stein Mill* **carbide blade**. Although the mass of stones is recorded, they are not considered in the "man-made inerts" class.

17.4 Sieve the < 4-mm fraction (from step 17.2.1) through a 2-mm sieve and analytically transfer materials that pass through the 2-mm sieve to a separate, clean and sterile sample container.

17.4.1 Transfer the remaining material (2-mm to 4-mm fraction) to a clean lab tray or other flat, smooth surface and remove all sharps and stones. Weigh and record the mass of the removed sharps and stones.

17.4.2 *Recombine Inert-free Size Fractions*—Analytically recombine the stone-free 2-mm to 4-mm sieve fraction with the < 2 mm fraction. Analytically recombine this with the 4-mm to 9.5-mm fraction. Transfer the recombined inert-free material to a clean storage container and seal for other tests, (e.g., metals, etc.).

17.5 *Sieve fractions > 9.5-mm*—These fractions include 9.5-mm to 16-mm, 16-mm to 25-mm, 25-mm to 50-mm, and > 50-mm. Steps outlined below may be repeated for each size fraction.

17.5.1 Place up to 250 cm^3 of material into a tared container. Obtain and record the gross as-received weight, (±0.01 g).

17.5.2 Dry the sample in a forced air oven at 70±5°C for 8 h to 24 h, until weight change due to moisture loss diminishes to nil. Obtain and record the gross oven-dry weight, (±0.01 g).

17.5.3 Place the entire oven-dried aliquot onto a clean tray.

17.5.3.1 Remove separately with a tweezers, all plastic, metal, glass, sharps, (e.g., sewing needles, straight pins and hypodermic needles, etc.), and

recalcitrant wood chips, (e.g., bulking agents and other organic materials not readily degraded during the composting process). Stone removal is not necessary unless the sample is to be milled.

17.5.3.2 Obtain and record the mass of each inert class.

17.5.4 *Optional*—Repeat steps under 17.5 for each sieve fraction. Air-dry and perform and inert counts for sieve fraction 9.5-mm to 16-mm, 16-mm to 25-mm, 25-mm to 50-mm, and > 50-mm.

18. Calculations for Method C

18.1 *Ratio of Inert Plastics, Metal and Glass to Sample by Size Fraction:*

$P_i = IP_i \div S_i$ *Equation 18.1.1*

$M_i = IM_i \div S_i$ *Equation 18.1.2*

$G_i = IG_i \div S_i$ *Equation 18.1.3*

$W_i = W_i \div S_i$ *Equation 18.1.4*

where:

i = sieve size fractions of interest,
IP = inert plastics weight, g,
IM = inert metals weight, g,
IG = inert glass weight, g,
W = wood chips, not to be considered as man-made inerts, g, and
S = mass of sample for size fraction "i", oven- or air-dried basis, (e.g., 70°C or 36°C), g.

18.1.1 Repeat calculations from step 18.1 for each sieve size fraction of interest, "i".

18.2 *Total Inerts by Size Fraction:*

$TI_i = [P_i + M_i + G_i] \times 100$ *Equation 18.2*

where:

TI = total inerts by size fraction of interest, "i", %,
i = sieve size fraction of interest,
P_i = ratio of plastics in test aliquot, unitless,
M_i = ratio of metals in test aliquot, unitless,
G_i = ratio of glass in test aliquot, unitless.

18.2.1 Repeat the calculation for each sieve size fraction of interest using Equation 18.2, "i".

18.3 *Total Inerts from Bulk Sample*—Multiply total inerts from step 18.2 by the ratio for each corresponding size fraction of interest, "i", *(from Method 02.02-B, Equation 14.2.1)*. Sum all fractions of interest.

$I_T = \Sigma [TI_i \times R_i] \times 100$ *Equation 18.3*

where:

I_T = total inerts in bulk sample, dry weight basis, 70±5°C, %,
TI = percentage of total inerts by sieve size fraction "i", (decimal fraction from Equation 18.2), unitless,
i = sieve size fractions of interest, and
R = ratio of each sieve size fraction "i", relative to bulk sample determined in the sieve test. Refer to Method 02.02-B, Equation 14.2.1.

18.4 *Total Recalcitrant Wood Chips*—Multiply ratio of wood chips (W) from step 18.1 by corresponding size fraction ratio *(from Method 02.02-B),* sum all fractions values.

$W_T = \Sigma (W_i \times R_i) \times 100$ *Equation 18.4*

where:

W_T = total recalcitrant wood chips relative to bulk sample, dry weight basis, %,
W = ratio of wood chips by sieve size fraction "i", (from Equation 18.1.4), unitless,
i = sieve size fractions of interest, and
R = ratio of each sieve size fraction "i", relative to bulk sample determined in the sieve test, unitless. Refer to Method 02.02-B, Equation 14.2.1.

Test Method: Laboratory Sample Preparation. Milling and Grinding Samples, Harrison Method						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
				02.02-D	02.02-D	02.02-D		02.02-D

02.02-D MILLING AND GRINDING SAMPLES, HARRISON METHOD

COMMENT—This method is based upon procedures developed for yard waste compost THAT DO NOT CONTAIN INERT CONTAMINANTS - *by Robert Harrison;* College of Forest Resources, University of Washington, Seattle, WA 98195.

CAUTION—This method is not recommended for use on highly heterogeneous samples nor on samples that require heavy metal determinations. Refer to 02.02-E for a preferred sample milling technique, i.e., a protocol similar to that commonly employed on plant tissue samples.

19. Apparatus for Method D

19.1 *Mill*—standard Wiley-type, large capacity, size #4.

19.2 *Mortar and Pestle*.

20. Reagents and Materials for Method D

20.1 *Water*—type II deionized, minimum resistivity of 17 MΩ·cm, minimum standard.

20.2 *Alconox*—mixed with tap water according to manufacturer's recommendations.

20.3 *Methanol.*

20.4 *Hexane*.

20.5 *10% HNO_3 Solution.*

21. Procedures for Method D

NOTE 2D—Generally, a minimum of 1 kg of compost is taken initially as a primary sample.

21.1 *Sample Drying*—For analysis of non-volatile compost components, compost is first dried at 70±5°C for 24 h in a drying oven. The sample is removed, allowed to cool for 1 h, weighed, and the weight recorded. The sample is then returned to the drying oven for an additional 24 h, removed, cooled and weighed again. If the sample weight changes less than 0.1% over the 24 h period, the sample is considered to be oven-dried at 70±5°C. Oven drying is not absolutely necessary, but makes the sample much easier to grind and equipment easier to clean.

21.1.1 This procedure is designed to remove primarily water adsorbed in compost pores. Some compost samples may contain substances that continue to volatilize over a period of time and continue to lose weight. In such cases, the samples should be dried for at least 24 h (2 cycles), and then the weight basis reported, (e.g., as oven-dried at 75°C for 48 h).

21.1.2 Some compost constituents of interest can be volatilized and lost by oven drying. Drying at 70±5°C is designed to reduce volatilization. When volatilization is considered to be a problem in sample analysis (volatile organic compounds, Hg, etc.), the samples should be ground and analyzed at as-received moisture or air-dried at 36°C, as soon as possible after sampling. Strive to minimize lag times between sampling, preparation and analysis. Moisture content is measured on a parallel sample to determine moisture content and oven dry mass of the sample analyzed.

21.2 *Wiley Mill Screen*—A 30-mesh or smaller screen (<0.5-mm) is placed in the Wiley mill. The screen should be clean and dry.

21.3 The compost is fed into the Wiley mill chamber slowly, removing large rock or metal pieces.

CAUTION—Care should be taken to avoid contamination of samples with dust during this process (Piper, 1942).

21.4 *Rocks and Stones*—If any rock particles are found, these are first ground to a powder with a mortar and pestle (if possible), and then introduced into the Wiley mill chamber.

COMMENT—This will dull the blades, but is necessary to produce a representative sample. Individual hard rock pieces can be set aside analyzed individually if desirable.

21.5 *Man-Made Inerts*—Any large hard metal fragments, (e.g., steel, etc.) cannot presently be analyzed. However, these are generally not found in compost. If metal fragments are found, they can be set aside and analyzed individually. Aluminum and steel cans and battery cases can generally be ground. However, it is advisable to snip them into smaller pieces if they are larger than 0.5 inch in size.

21.6 *Repeat Milling Steps 3*×—The entire compost sample is collected and thoroughly mixed by running it through the Wiley mill a second and a third time.

21.7 *Clean Wiley Mill*—All surfaces contacting compost are cleaned by scrubbing with Alconox and tap water, rinsed with tap water, rinsed with methanol, rinsed with hexane, rinsed with 10% HNO_3 solution, rinsed with reagent grade water, and air dried.

21.8 Repeat the above grinding procedure for additional samples.

Test Method: Laboratory Sample Preparation. Milling and Grinding Samples, Munter Method						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
				02.02-E	02.02-E	02.02-E		02.02-E

02.02-E MILLING AND GRINDING SAMPLES, MUNTER METHOD

NOTE 1E—This method emulates the protocol commonly employed on plant tissue samples and is based on sample preparation procedures developed by Robert Munter for mixed municipal solid waste (MSW) compost; University of Minnesota Research Analytical Laboratory, Department of Soil, Water and Climate, St. Paul.

22. Apparatus for Method E

22.1 *Plant Tissue Mill*—equipped with tool steel blade with 1 in carbide cutting edge and aluminum mill cup (e.g., Stein Mill, model M-2, 15,000 rpm, ½ HP. Fred Stein Laboratories, Inc., 121 N. 4th St., Atchinson, KS 66002).

22.2 *Bottle*—plastic, 250- or 300-mL, wide mouth.

23. Reagents and Materials for Method E

23.1 *None Required.*

24. Procedure for Method E

ATTENTION—A 250-mL aliquot of an air-dried working sample is used for milling. The sample should be free from inerts and stones > 2 mm. Stones are removed from the sample to protect the carbide blades of the mill, and for consistency of results.

24.1 *Milling Cup Preparation:*

24.1.1 Fill the aluminum mill cup to about one fourth to one third capacity.

24.1.2 Lock in cup so it fits tightly in the position to engage the safety switch.

24.1.3 *Milling Time*—1 min of milling time is adequate for achieving finely ground samples. Additional milling contributes to erosion of metal from the steel/carbide blade.

24.2 *Mill Sample*—Turn the switch to *ON*.

24.3 *Milled Sample Storage*—Transfer the sample to a wide mouth plastic bottle for storage and chemical analysis: organic carbon, total nutrients (not including N), and heavy metals.

24.4 *Clean Mill*—It is imperative to remove all sample residues from the mill and sample cup before processing the next sample.

Note 2E—Determinations for TN (optional), NO_3, NH_4, pH, electrical conductivity, other water-soluble elements, biological assays, respirometry, and pathogens screening are performed on as-received moist compost test aliquots; milling may not be practical with most as-received moist compost samples.

Test Method: Laboratory Sample Preparation. Feedstock Laboratory Sample Preparation Modifications						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	02.02-F	02.02-F						

02.02-F MODIFICATIONS FOR FEEDSTOCK SAMPLE PREPARATION

25. Significance of Method F

25.1 Feedstock sample fragment sizes and sample heterogeneity are generally much larger than those of refined compost materials. For these reasons, laboratory preparation methods must be slightly modified to compensate for these differences.

25.2 As with a compost sample, feedstock sample heterogeneity is significantly decreased by thorough milling or grinding of a large sample. This technique improves the precision of elemental and chemical analyses by homogenizing the sample aliquot selected to represent the original feedstock blend. Mill and homogenize at least 1000 cm^3 of the feedstock sample.

25.3 Volatile loss of nutrients and other compounds may become significant with excessive handling during sample homogenization. When volatile losses are an issue, sample preparation shall be performed in a cold room with a temperature of no more than 4°C to minimize volatile losses.

26. Sample Handling and Procedural Modifications for Feedstocks

26.1 *Method 02.02-A Sample Mixing and Splitting*—No Modification. Follow procedures as outlined.

26.2 *Method 02.02-B Sample Sieving for Aggregate Size Classification*—Modifications Required. Follow procedures as outlined.

26.2.1 *Working Sample*—After completing all steps and recording all measurements.

26.2.1.1 Merge all size classes of feedstock materials.

26.2.1.2 Thoroughly blend sample as outlined in 02.02-B.

26.2.1.3 Subdivide sample by quartering until approximately 1 L of material is isolated.

26.2.1.4 Follow storage procedure as outlined.

26.3 *Method 02.02-C Man Made Inert Removal and Classification:*

26.3.1 Increase feedstock aliquot size for inert removal to approximately 1,000 cm^3 and follow the procedure as outlined.

26.3.2 Modify the procedures as necessary to compensate for the increased sample size. Document and report all extraordinary procedural modifications.

26.4 *Methods 02.02-D and 02.02-E Milling and Grinding Sample:*

26.4.1 Increase feedstock aliquot size for milling to at least 1,000 cm^3 (1 L) and follow the procedure as outlined.

26.4.2 Modify the procedures as necessary to compensate for the increased sample size. Document and report all extraordinary procedural modifications.

02.02 SUMMARY

27. Report

27.1 *Method 02.02-A Sample Mixing and Splitting*—Report the mass of material received and its moisture content, wet basis.

27.2 *Method 02.02-B Sample Sieving for Aggregate Size Classification*—Report the percentage of each sieve fraction considered, % g g^{-1}, and the moisture content of each fraction.

27.3 *Method 02.02-C Man Made Inert Removal and Classification*—Report inerts relative to the bulk sample, oven dried weight basis (70±5°C), % g g^{-1}.

27.3.1 Report percentages of inerts by inert type relative to the bulk oven-dried sample.

27.3.1.1 Combined film plastics and hard plastics are reported as plastics without differentiating plastic type. Film plastics are reported as unit area of film plastics per unit volume of compost, cm^2 m^{-3}. Refer to Method 03.05-A Film Plastic Surface Area Determinations Using Digital Processing.

27.3.1.2 Percent recalcitrant wood chip is reported as a percentage on an oven dried weight basis, % g g^{-1}.

27.3.2 Report sum total inerts as a percentage of the bulk oven-dried sample.

27.4 *Method 02.02-D Milling and Grinding Samples, Harrison*—Report method selected for milling, drying temperature and time (°C·h), sieve size fractions included in the milled sample, and the inert content of the milled sample.

27.5 *Method 02.02-E Milling and Grinding Samples, Munter*—Report method selected for milling, drying temperature and time (°C·h), sieve size fractions included in the milled sample, and the inert content of milled sample.

27.6 *Method 02.02-F Modifications for Feedstock Sample Preparation*—Report material type(s), method selected for milling, sieve size distribution, inert content of sample by fraction. Refer to specific method for details.

28. Precision and Bias

28.1 *Inert Plastics, Metal, Glass and Total Inerts:*

28.1.1 *Method 02.02-C Man Made Inert Removal and Classification*—Precision of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

28.1.1.1 Precision was determined using 10 subsamples taken from a field composite sample for each of three mixed municipal solid waste composting (MSW) facilities for two sampling periods in 1993.

Table 02.02-C1 Precision estimates for man-made inerts, (%CV for plastics, metal, glass), in <6.3 mm air-dried mixed municipal solid waste compost, 1993.

Site	*Plastics*	*Metal*	*Glass*	*Number of Samples*
A	25	186	47	10
B	30	0	33	10
C	29	160	166	10
A	25	142	94	10
B	35	285	190	10
C	28	155	316	10

29. Keywords

29.1 milling; grinding; sieving; inerts; plastics; metal; glass; stones; rocks; sieve size; particle size; moisture; oven-dried; air-dried; as-received; sample splitting; sharps

Test Method: Air Capacity. Three Methods.						**Units:** % v v^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	03.01-A	03.01-A	03.01-A	03.01-A				
		03.01-B		03.01-B		03.01-B		
	03.01-C	03.01-C	03.01-C	03.01-C		03.01-C		

03.01 AIR CAPACITY

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the measurement of moisture content, volume weights (bulk density), porosity, water-holding capacity, and air capacity (free air space) of compost materials.

1.1.1 *Method 03.01-A Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Unsieved)*

1.1.2 *Method 03.01-B Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Sieved)*

1.1.3 *Method 03.01-C Field Density, Free Air Space and Water-Holding Capacity*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 02.01-B Selection of Sampling Locations for Windrows and Piles

Method 02.01-D Batch Feedstock Material Sampling Strategies

Method 03.09-A Total Solids and Moisture at 70±5°C

2.2 *Other Sources:*

ASTM D 2980-71, Standard Test Method for Volume Weights, Water Holding Capacity, and Air Capacity of Water-Saturated Peat Materials. *In* Annual Book of ASTM Standards, Vol. 04.08. (Re-approved 1990).

Haug, Roger T., The Practical Handbook of Compost Engineering, Lewis Publishers, 1993.

Water holding Capacity, Volume Mass and Air Capacity of Water-Saturated Peat. ASTM D 2989-71. p 77. *In* Peat Testing Manual. National Research Council of Canada, Technical Memorandum No. 125. 1979.

Laboratory Procedure for the Preparation of Solid Waste and Related Materials for Analysis. p 3. *In* Methods of Solid Waste Testing. 1973. US EPA. Office of Research and Monitoring. US EPA-6700-73-01, Part I.

3. Terminology

3.1 *air capacity, n*—Proportion of the bulk volume of compost, finished planting media, or soil that is filled with air at any given time or under a given condition. Compost, finished planting media, or soil with high air capacity has the capacity to hold more water. Air capacity is important in field application because it is related to soil reconsolidation rates. Air capacity is indicates the ability of a compost to resist water logging and low oxygen levels.

3.2 *bulk density, n*—Weight per unit volume of compost, calculated and reported on an oven dry weight basis, 70±5°C, w v^{-3}.

3.3 *free air space, n*—Air-filled pore volume of an as-received compost material, % v v^{-1}.

3.4 *porosity, n*—Sum of water-filled pore volume plus air-filled pore volume, cm^3.

3.5 *pore space, n*—Sum of water-filled pore volume plus air-filled pore volume relative to the overall volume of the compost, % v v^3.

3.6 *water holding capacity, n*—Percentage of water filled pore volume relative to the total volume of water saturated compost, % w w^{-1}.

3.7 *equivalency, n*—at STP, 1 g of deionized water ≡ 1 cm^3 of deionized water ≡ 1 mL of deionized water ≡ 1 mL of air space ≡ 1 cm^3 of air space.

4. Summary of Test Methods

4.1 *Method 03.01-A Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Unsieved)*—A compost sample taken from the pile, as is, of known volume and mass is transferred to a graduated beaker and bulk density is determined. The compost is saturated with water and excess water is drained. Changes in compost volume and mass, and the ratio of water retained relative to the amount of drained water provide a means for estimating compost bulk density, porosity/pore water volumes and free airspace, and water holding capacity.

4.2 *Method 03.01-B Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Sieved)*—A compost sample taken from the pile, as is, of known volume, is sieved and systematically transferred to a graduated beaker and bulk density is determined. The compost is saturated with water and excess water is drained. Changes in compost volume and mass, and the ratio of water retained relative to the amount of drained water provide a means for estimating compost bulk density, porosity/pore water volumes and free airspace, and water holding capacity.

4.3 *Method 03.01-C Field Density, Free Air Space and Water-Holding Capacity*—Compost samples are systematically transferred to a bucket and weighed to approximate compost density. The bucket containing the compost is filled with water and weighed; where the volume equivalent of the added water is used to approximate compost pore space. Then, the water is drained and the remaining water-saturated compost is weighed to approximate the water-holding capacity of the compost.

5. Significance and Use

5.1 When large air spaces are present, high water penetration and aeration can occur. If average air space dimension diminishes and total air space remains unchanged, retention of water increases and the potential for water penetration or flow decreases. Water retention is greatest in humified materials that have small air spaces and low bulk density, whereas water penetration and aeration is greater in poorly humified compost with correspondingly larger air spaces. Percent air space in compostable mixtures needs to be great enough to allow for maintenance of aerobic condition.

5.1.1 Free airspace for composting should be greater than 60% initially, and at least 35% during curing. Free airspace less than 60% initially and 35% during curing inhibits air flow through the pile and will result in accumulation of carbon dioxide and consequent formation of anaerobic conditions; the latter lead to odors from volatile organic acids, sulfides, and amines. formation. Free airspace during odor treatment, if a biofilter is used, should be about 80-90%.

5.2 *Method 03.01-A Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Unsieved)*—Quick-Test to determine volume weights (bulk density), porosity/pore space, water-holding capacity, and air capacity (free air space) performed on unsieved, as-received compost.

5.2.1 This method provides a quick estimate for compost bulk density, porosity/pore space percent, free airspace percent, and water holding capacity. Approximately 5 h are required to complete a run, whereas 24 h are required to perform the method described under *Method 03.10-D Bulk Density, Water-Holding Capacity, and Air Capacity of Compost Material, Modified ASTM D 2980-71.*

5.3 *Method 03.01-B Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Sieved)*—Quick-Test to determine volume weights (bulk density), pore space, water holding capacity, and air capacity (free air space) performed on sieved, as-received compost material.

5.3.1 This method is identical to *Method 03.01-A* with exception of an additional step in sample preparation which requires sample sieving (9.5-mm sieve).

5.4 *Method 03.01-C Field Density, Free Air Space and Water-Holding Capacity*—Field test using a typical bucket provides an inexpensive, quick approximation of density and porosity for feedstock blends and in-process composting material for process management. This field procedure is useful for evaluating the need for bulking agent.

6. Interference and Limitations

6.1 In-process compost sample moisture should range from 45-60% (wet basis). Finished product compost sample moisture should range from 40-50% (wet basis). Excessively moist samples will compact during preparation, and inflate bulk density estimates and deflate percent free air space estimates. Excessively dry samples are often difficult to saturate with water (sometimes hydrophobic) and may result in over-estimates of percent free air space.

CAUTION !—Excessively moist or dry initial in-process compost samples may yield invalid results.

6.2 Three of the 03.01 Methods (A, B, C) do not use vacuum to assist water extraction from water filled pores (*c.f.* Method 03.10-D). Therefore, incomplete

removal of free water *(water-filled air space)* from air pore space will deflate air capacity estimates and inflate water-holding capacity estimates.

6.3 6.3 *Method 03.01-C Field Density, Free Air Space and Water-Holding Capacity*—This method uses large aliquots and should be used as a rough guide to generate approximations of sample bulk density, free airspace, and water-holding capacity.

7. Sample Handling

7.1 *Method 03.01-A Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Unsieved)*—Material used in this test should represent in-process compost product at 45-60% moisture (wet weight basis). The sample aliquot should be unsieved, as-received.

7.2 *Method 03.01-B Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Sieved)*—Material used in this test should represent in-process compost product at 45% - 60% moisture (wet weight basis). The sample aliquot should be sieved through a 9.5-mm sieve, as-received.

7.3 *Method 03.01-C Field Density, Free Air Space and Water-Holding Capacity*—Material used in this test should represent in-process compost or feedstock blends, moistened to attain 45-60% moisture content (wet weight basis). The sample aliquots should not be sieved, but represent the particle and fragment size distribution of the in-process materials in question.

Test Method: Air Capacity. *Quick-Test* for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost						**Units:** % v v^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	03.01-A	03.01-A	03.01-A	03.01-A				
		03.01-B		03.01-B		03.01-B		

03.01-A AND 03.01-B QUICK-TEST FOR BULK DENSITY, POROSITY/PORE SPACE, FREE AIRSPACE AND WATER-HOLDING CAPACITY OF COMPOST

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTED BY—This method is based upon procedures developed for composting process monitoring and finished compost by P.B. Leege and Melinda Miller.

8. Apparatus for Methods A and B

8.1 *Graduated Cylinder*—two 1000-mL, for water addition.

8.2 *Graduated Beaker with Handle*—two 2000-mL.

8.2.1 Low-form polypropylene, straight-wall (not tapered).

8.2.2 Low-form polypropylene, straight-wall (not tapered), modified in the lab with four 0.3 mm (⅛ in.) diameter holes drilled uniformly spaced through the bottom of the beaker at the low concave spots to promote uniform drainage from the beaker's contents.

8.3 *Analytical Balance*—top loading, high capacity, accurate to ±0.1 g with 0.1-1 kg range.

8.4 *Drying Oven*—forced air.

8.5 *Beaker*—150-mL, Pyrex.

8.6 *Desiccator with Desiccant.*

8.7 *Rubber Mat*—closed-cell polyethylene foam mat, (4) 0.6-mm (¼-in.) layers stacked.

8.8 *Funnel*—approximately 2.5-cm (~1-in.) diameter delivery stem, 15 cm (6 in.) mouth.

8.9 *Pan*—25-cm (10 in.), stainless steel or brass (sieve catch beaker or equivalent).

8.10 *Sieve*—stainless steel, 4 or 5 cm mesh (1.5 or 2 in.), or equivalent metal platform.

8.11 *Timer*—with alarm.

8.12 *Watch glass*—5-cm (2-in.) diameter, or parafilm to cover beaker and graduate cylinder.

9. Reagents and Materials for Methods A and B

9.1 *Water*—type II deionized, 17 MΩ·cm minimum resistivity.

9.2 *Adhesive Tape*—2.5 cm (1 in.) width masking, or equivalent.

10. Procedure for Methods A and B

10.1 *Sample Oven Dry Weight Determination:*

10.1.1 Weigh and record tare weight of dry, open 150-mL beaker using analytical balance, ±0.1 g.

10.1.2 Transfer a 50 cm^3 aliquot of a parallel sample to a tared 150-mL open beaker.

10.1.3 Weigh and record the 50 cm^3 aliquot gross as-received weight (as received moisture) using analytical balance, ±0.1 g.

10.1.4 Subtract open tare weight of dry open 150-mL beaker from 50 cm^3 aliquot as-received gross weight, ±0.01 g, (designate as A_{50}).

10.1.5 Oven dry the 50 cm^3 aliquot in a forced air oven set at 70±5°C for 18-24 h. Weigh and record the oven-dry weight of 50 cm^3 aliquot.

10.1.6 Subtract the tare weight of the dry, open 150-mL beaker from the gross oven dry weight of the 50 cm^3 aliquot to determine sample net oven dry weight, ±0.01 g, (designate as O_{50}).

10.2 *Modified Graduated Beaker Preparation:*

10.2.1 Drill four 3-mm (⅛-in.) uniformly spaced holes in the bottom of one 2000-mL beaker.

10.2.2 Temporarily cover and seal the drain holes of the modified 2000-mL graduated beaker from the bottom side with 2.5 cm (1 in.) wide masking tape . Press tape down securely to prevent leakage.

10.3 Weigh and record the tare weight of the taped, dry, empty modified 2000-mL graduated beaker, ±0.01 g.

10.4 *Fill Graduated Beaker with Compost:*

10.4.1 Transfer a 600 cm^3 aliquot of as-received compost into the modified 2000-mL graduated beaker through the funnel.

NOTE 1A—To ensure uniform packing of compost throughout the modified graduated beaker, allow beaker containing

compost to fall freely onto a rubber mat once from height of 15 cm (6 in.). Carefully maintain the beaker in an upright position at all times.

10.4.2 Repeat the filling with 600 cm^3 and free falling operation, two more times (three times total). After the third free-fall drop, fill the graduated beaker to volume with sample material, 1800 mL. Do not repeat free-fall drop after topping off. Topping off should be limited to 2-3 cm.

10.5 *As-Received Sample Weight Determination:*

10.5.1 Weigh and record gross weight of taped, modified 2000-mL graduated beaker containing 1800 cm^3 of as-received compost, ±0.01 g.

10.5.2 Subtract initial tare weight of taped, modified 2000-mL graduated beaker from weight of taped, modified 2000-mL graduated beaker containing 1800 cm^3 as-received compost, (designated as A_{1800}).

10.6 *Saturate Sample with Water:*

10.6.1 Saturate compost sample slowly and carefully by pouring deionized water onto compost in the graduated beaker with 1000-mL graduated cylinder.

10.6.2 Continue pouring until the top face of compost in the 2000-mL graduated beaker glistens with free water; be careful not to add excess water.

10.6.3 Cover the modified 2000-mL graduated beaker using a watch glass or parafilm and allow wetted compost sample to rest for approximately 5 min.

10.6.4 Repeat water addition with 5-min rest periods until water is no longer absorbed and the compost surface glistens with a film of free water. Do not add excess water; avoid pooling at sample surface.

10.6.5 Read and record volume of water required to saturate compost sample from 1000-mL graduated cylinder, ±5 mL.

10.6.6 Set aside 1000-mL cylinder with any remaining water for later use.

10.7 *Pore Space Estimate:*

10.7.1 Place modified graduated beaker containing water saturated compost upright onto a 3.8 or 5 cm ($1^1/_2$ or 2 in.) grate atop the unmodified 2000-mL graduated beaker with handle, or equivalent setup to catch drainage from bottom of the modified 2000-mL graduated beaker.

10.7.2 Holding the modified 2000-mL graduated beaker over the water catch stand, remove masking tape from bottom of the beaker to allow water to drain from the saturated compost beaker into catch beaker.

10.7.3 Capture drained water in catch beaker for reuse.

10.7.4 After 30 min cover drain holes with masking tape. Slowly and carefully saturate compost sample by pouring water from catch beaker back onto compost filled modified 2000-mL graduated beaker.

NOTE 2A—Do not add excess water, avoid water pooling at sample surface.

10.7.5 Repeat this operation at least three times using the captured drainage water to ensure that compost sample air spaces fill with water during wetting process.

10.7.6 Add small volumes of as-received deionized water from 1000 mL cylinder (see step 10.6.6) as necessary if recycled drainage water from catch beaker fails to re-saturate sample.

10.7.7 Set aside the covered catch beaker without drying for later use.

NOTE 3A— Previously unfilled pores will fill with water. The total volume of water needed to saturate sample represents sample pore space. After saturation is reached, no pockets of trapped air should be visible.

NOTE 4A—Carefully monitor volume of water needed to re-fill modified graduated beaker. During refilling, systematic water loss will occur. The amount of water lost may be minimized by using very clean equipment.

NOTE 5A—Sample compaction may occur with mature composts. If recycled drainage water is not reabsorbed after allowing a saturated sample to rest, subtract excess volume of water from initial water volume needed to saturate compost. Avoid pooling water at sample surface. When excess water is retained to avoid over saturation of sample, subtract volume of excess deionized water (from Step 10.7) from initial volume of water (from Step 10.6). Sample expansion may occur with immature, unstable composts. If recycled drainage water is completely reabsorbed after allowing saturated sample to rest, add additional water to saturate compost sample. Add water to initial water volume to saturate compost sample. When water is needed to saturate sample, sum volume of added (from Step 10.7) and initial volume of water (from Step 10.6).

10.8 *Water-Saturated Weight and Volume before Draining:*

10.8.1 Weigh and record gross weight of taped, modified graduated beaker containing water and water-saturated compost, ±0.01 g.

10.8.2 Subtract initial tare weight of taped, graduated beaker from weight of taped, modified graduated beaker containing water and water-saturated compost, ($W_{1800\ WS,\ no\ drain}$), g.

10.8.3 Read and record the volume of water-saturated compost in beaker, (designate as $V_{1800WS,\ no\ drain}$), mL.

NOTE 6A—Verify total volume of water added (from Step 10.7) by comparing the difference between initial sample weight (from Step 10.5) and sample weight at saturation (from Step 10.8).

10.9 *Drain Cylinder:*

10.9.1 Drain off water into catch beaker for the last time through drainage holes and allow sample to rest and continue to drain for 4 h.

10.9.1.1 Cover the modified 2000-mL graduated beaker to minimize evaporative water loss during the 4 h final drain period.

10.9.1.2 Capture drainage water in the catch beaker.

10.9.2 Read and record net volume of drainage water released from compost during the 4-h draining period, ±0.01 g, combined with water already in catch beaker.

10.10 *Determine Water-Saturated Weight After 4-h Draining:*

10.10.1 Weigh and record the mass of taped, modified graduated beaker and water-saturated compost, ±0.01 g.

10.10.2 Subtract initial tare weight of graduated beaker from the weight of beaker containing water-saturated compost, g. (designate as $W_{1800\ WS,\ after\ drain}$)

NOTE 7A—Compare and verify free airspace volume determination by dividing volume of drainage water (from Step 10.9) by total volume of water added to saturate compost sample (from Steps 10.7 and 10.8).

10.11 *Water-Saturated Volume*—Read and record volume of wetted compost in modified graduated beaker, ±5 mL.

10.12 *Perform Calculations.*

11. Calculation for Methods A and B

11.1 *Sample Oven Dry Weight (O_{1800}):*

$$O_{1800} = A_{1800} \times (O_{50} \div A_{50}) \qquad \textit{Equation 11.1}$$

11.2 *Moisture Content (M_{1800}):*

$$M_{1800} = A_{1800} \times [1 - (O_{50} \div A_{50})], \text{ or} \qquad \textit{Equation 11.2.1}$$

$$M_{1800} = A_{1800} - O_{1800} \qquad \textit{Equation 11.2.2}$$

11.3 *Bulk Density (BD):*

$$BD = O_{1800} \div V_{1800} \qquad \textit{Equation 11.3}$$

11.4 *Pore Space Volume (PSV)*

$$PSV = W_{1800\ WS,\ nd} - O_{1800} + (1800 - V_{1800\ WS,\ no\ drain}) \qquad \textit{Equation 11.4}$$

NOTE 8A—Assume that 1 g of deionized water ≡ 1 cm^3 of deionized water ≡ 1 mL of deionized water ≡ 1 mL of air space.

11.5 *Pore Space Percent (PS, %):*

$$PS = PSV \div 1800 \times 100 \qquad \textit{Equation 11.5}$$

11.6 *Free Airspace Volume, Mass Equivalent Method (FASVme):*

$$FASVme = W_{1800\ WS,\ nd} - W_{1800\ WS,\ after\ drain} \qquad \textit{Equation 11.6}$$

11.7 *Free Airspace Percent (FAS, %):*

$$FAS = FASVme \div V_{1800} \times 100 \qquad \textit{Equation 11.7}$$

11.8 *Water Holding Capacity (WHC):*

11.8.1 Percent water holding capacity, volume basis:

$$WHC_V = PS - FAS \qquad \textit{Equation 11.8.1}$$

11.8.2 Percent water holding capacity, mass basis:

$$WHC_M = [W_{1800\ WS,\ ad} - O_{1800}] \div O_{1800} \times 100 \qquad \textit{Equation 11.8.2}$$

where:

A_{50} = as-received sample weight of 50 mL aliquot (as received), g, (step 10.1.4),

O_{50} = oven dry weight of 50 mL aliquot, g, (step 10.1.5),

A_{1800} = initial weight of 1800 cm^3 (mL) sample (as received), g, (step 10.5.2),

O_{1800} = calculated oven dry weight of 1800 cm^3 (mL) sample, g, (step 11.1),

$W_{1800\ WS,\ nd}$ = weight of water-saturated compost *before* final 4 h draining, g, (step 10.8.2),

$W_{1800\ WS,\ ad}$ = weight of water-saturated compost *after* final 4 h draining, g, (step 10.10.2),

V_{1800} = initial volume of compost in cylinder (1800 cm^3), mL ≡ cm^3,

$V_{1800\ WS,\ nd}$ = volume of water-saturated compost *before* final 4 h draining, g, (step 10.8.3),

PSV = pore space volume, mL,

$FASV_{me}$ = free airspace volume using mass equivalents, 1 mL ≡ 1 g,

$FASV_{vm}$ = free airspace volume using volume measures, 1 mL ≡ 1 cm^3,

BD = bulk density, g cm^{-3},

PS = pore space, % v v^{-1},

FAS = free airspace, % v v^{-1},

WHC_V = water holding capacity, % v v^{-1}, and

WHC_M = water holding capacity, % w w^{-1}.

Test Method: Air Capacity. Field Density, Free Airspace and Water-Holding Capacity						**Units:** *See Calculations*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
03.01-C	03.01-C	03.01-C	03.01-C	03.01-C	03.01-C	03.01-C		

03.01-C FIELD DENSITY, FREE AIRSPACE AND WATER-HOLDING CAPACITY

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

12. Apparatus for Method C

12.1 *pail*—20-L (5-gal), plastic with lip and vertical walls, fitted with hoop-type handle.

12.2 *scale*—20 kg, accurate to ±50 g.

12.3 *rule*—tape measure or ruler.

12.4 *adhesive tape*—1.3 cm (½ in.) width, brightly colored such as blue masking tape.

12.5 *marking pen*—dark colored.

12.6 *surface*—firm, flat, such as cement or pavement.

12.7 *cheese cloth*—60 x 60 cm (24 x 24 in.) square, or other equivalent material to serve as a strainer or sieve.

12.8 *strap*—90-cm (~3-ft) segment of wire, rope or cord to secure strainer or sieve over mouth of pail.

12.9 *grate*—to facilitate unobstructed drainage of pail.

12.10 *graduated cylinder*—1000-mL, plastic or glass.

13. Reagents and Materials for Method C

13.1 *water*—20 L (5 gal), tap water

14. Procedure for Method C

14.1 Collect a composite sample of compost as described in TMECC 02.01-B Selection of Sampling Locations for Windrows and Piles, or blended feedstocks as described in TMECC 02.01-D Batch Feedstock Material Sampling Strategies.

14.1.1 When performing this test on a feedstock blend, be sure to thoroughly mix the feedstocks before collecting a composite sample.

14.1.2 Determine total solids content on a parallel sample aliquot of the test material as described in TMECC 03.09 Total Solids and Moisture at 70±5°C.

NOTE 1C—It may be acceptable to dry the parallel sample aliquot at 105°C to decrease the required drying time. Absolute accuracy of total solids content is not always critical for process management.

14.2 *Preparation of Equipment:*

14.2.1 Subdivide the pail into three equal volumes. Measure from the inside bottom to the top rim of the pail; make a series of four or five marks spaced around the inside circumference of the pail with the marking pen to highlight each of two equally-spaced divisions; refer to the illustration in Fig 03.01-C.

14.2.2 Place a band of brightly colored tape over each of the two highlighted divisions on the inside circumference of the pail.

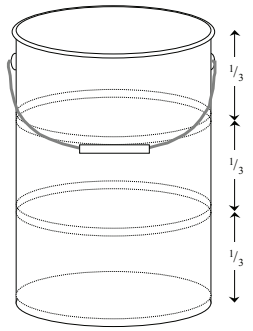

Fig 03.01-C Sample pail with three equal subdivisions.

14.2.3 Obtain the tare weight of the pail. Measure and record the dry mass of the empty pail.

14.2.4 Determine volume capacity of the pail (m^3). Fill the pail to the brim with water. Measure and record the weight of the water-filled pail. Alternatively, fill the pail using the 1000 graduated cylinder and record the volume of water used.

ASSUMPTION—1000 L of H_2O ≡ 1000 kg of H_2O ≡ 1.000 m^3 of H_2O; 1 Lb of H_2O ≡ 5.94×10^{-4} yd^3 ≡ 4.54×10^{-4} m^3

14.3 *Bulk Density:*

14.3.1 Transfer a first aliquot of material from the composite sample to fill the lowest third of the pail to the lower band of tape.

14.3.2 Systematically compact the sample aliquot. Drop the sample pail onto a firm flat surface ten times from a height of 15 cm (6 in.). Using the pail handle, carefully guide each 15-cm pail-drop to ensure that the pail remains upright and does not tilt or tip upon impact.

14.3.3 Transfer a second aliquot of material from the composite sample to fill the second third of the pail to the upper band of tape; repeat the compaction procedure (step 14.3.2).

14.3.4 Transfer a third aliquot of material from the composite sample to fill the pail to the brim; repeat the compaction procedure (step 14.3.2).

14.3.5 Transfer a forth and final aliquot of material from the composite sample to fill the pail to the brim; the pail should be completely filled with sample material; DO NOT COMPACT THE FORTH ALIQUOT.

14.3.6 Measure and record the mass of the filled pail.

14.4 *Free Air Space:*

14.4.1 *Mass based Method*—Fill the pail containing the density sample (from step 14.3) with tap water.

14.4.2 *Alternate Volume based Method*—Transfer tap water into the compost-filled pail using the 1000 mL graduated cylinder. Record the volume water required to fill the pail to the brim.

14.4.3 Measure and record the mass of the compost/water filled pail.

14.5 *Water-Holding Capacity:*

14.5.1 Allow the sample to rest for three h, to allow the tap water to absorb into the moist, compacted sample material.

14.5.2 Cover the mouth of the pail with cheese cloth or other material to function as a filter and securely fasten the filter over the lip of the pail mouth.

14.5.3 Invert the water-filled pail onto the elevated grate to drain free water from the saturated material. Allow to drain for 24 hr.

14.5.4 Remove the filter cloth; measure and record the combined mass of the pail and water-saturated compost.

15. Calculations for Method C

15.1 *Bulk Density Estimate at Field Moisture:*

$$A = [(B - C) \div (D - C)] \qquad \textit{Equation 15.1}$$

where:

A = bulk density estimate, kg m^{-3} dw (Lb yd^{-3}) basis,
B = mass of the compost-filled pail (step 14.3.6), kg (Lb),
C = mass of the empty pail (step 14.2.3), kg (Lb), and
D = volume of the water-filled pail (step 14.2.4), m^3 (yd^3).

CONVERSION FACTORS:
$D_{Imperial\ 5\text{-}gal}$ = 0.022730 m^3, and
$D_{US\ 5\text{-}gal}$ = 0.024755 yd^3.

ASSUMPTION—1000 L of $H_2O \equiv$ 1000 kg of $H_2O \equiv$ 1.000 m^3 of H_2O; 1 Lb of $H_2O \equiv 5.94\times10^{-4}$ $yd^3 \equiv 4.54\times10^{-4}$ m^3

15.2 *Free Air Space Estimate by Weight at Field Moisture:*

$$A = [(B - C) \div (B - D)] \times 100 \qquad \textit{Equation 15.2}$$

where:

A = pore space, w w^{-1}, %,
B = mass of the water/compost-filled pail (step 14.4.3), kg (Lb)
C = mass of compost-filled pail (step 14.3.6), kg (Lb), and
D = mass of the empty pail (step 14.2.3), kg (Lb)

15.3 *Free Air Space Estimate by Volume:*

$$A = (B \div C) \times 100 \qquad \textit{Equation 15.3}$$

where:

A = free air space, v v^{-1}, %,
B = volume of water added to compost-filled pail (from step 14.4.1 or 14.4.2), mL, and
C = volume of water-filled pail (step 14.2.3), mL

CONVERSION FACTORS:
$C_{Imperial\ 5\text{-}gal}$ = 22.73046 L, or 22730 mL, and
$C_{US\ 5\text{-}gal}$ = 18.92706 L, or 18927 mL.

15.4 *Water-Holding Capacity Estimate by Weight:*

$$A = [(B - C) - (D - C) \times E] \div [(D - C) \times E] \qquad \textit{Equation 15.4}$$

where:

A = water-holding capacity, kg kg^{-1} (Lb Lb^{-1}), %,
B = mass of the water-saturated compost-filled pail (step 14.5.4), kg (Lb),
C = mass of empty pail (step 14.2.3), kg (Lb),
D = mass of the compost-filled pail (step 14.3.6), kg (Lb), and
E = total solids ratio (step 14.1.2), unitless.

03.01 METHODS SUMMARY

16. Report

16.1 *Free Air Space*—Express free air space as a percentage, volume of free air space per unit volume of compost (%, v v, ±0.1 %).

16.2 *Bulk Density*—Express bulk density as mass per unit volume of compost on an as-received moisture basis to the nearest ±0.1 w v^{-3} (kg m^3, or Lb yd^3).

16.3 *Water-Holding Capacity*—Express water-holding capacity as a percentage of the volume (mass equivalent) of water retained per unit mass of compost (dw basis) to the nearest ±0.1 %, w w^{-1}.

16.4 *Moisture Content or Total Solids*—Report as-received moisture or total solids content as a percentage, %, w w^{-1}, wet weight basis as determined by forced-air oven-drying at 70±5°C.

17. Precision and Bias

17.1.1 *Air Capacity, Bulk Density, Water-Holding Capacity:* The precision and bias of the tests (Methods 03.01 A, B, C) have not been determined. Data are being sought for use in developing a precision and bias statements.

18. Keywords

18.1 air capacity; air space; bulk density; free airspace; porosity; pore space; water-holding capacity; field density; field porosity; field test; bucket test

Test Method: Ash. Three Methods.						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	03.02-A	03.02-A		03.02-A		03.02-A		
				03.02-B		03.02-B		
								03.02-C

03.02 ASH

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the measurement of ash content and volatile solids content for compost materials and feedstocks.

1.1.1 *Method 03.02-A Unmilled Material Ignited at 550°C without Inerts Removal.*

1.1.2 *Method 03.02-B Milled Material Ignited at 550°C with Inerts Removal.*

1.1.3 *Method 03.02-C Unmilled Material Ignited at 550°C with Inerts Removal.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 03.09-A Total Solids and Moisture.

Method 05.01 Biodegradable Volatile Solids.

Method 05.07-A Loss-on-Ignition Organic Matter.

2.2 *Other References:*

Cohen, I.R. 1973. Laboratory Procedure for the Preparation of Solid Waste Related Materials for Analysis. *In* Methods of Solid Waste Testing, EPA-6700-73-01. US EPA, Cincinnati, OH.

Methods for the Evaluation of Water and Wastewater, EPA 0600/4-79-020, US EPA, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

SM 2540-E, Fixed and Volatile Solids Ignited at 500°C. *In* Standard Methods for the Examination of Water and Wastewater. Part 2000, Physical and Aggregate Properties. 18th edition. 1992.

US EPA Method 600/4-79-020, adapted by physical removal of volatile solids that are not readily biodegradable.

3. Terminology

3.1 *ash, n*—The inorganic matter, or mineral residue of total solids that remains when a compost or feedstock is combusted at 550°C in the presence of excess air; equivalent to *fixed solids*, % g g^{-1}.

3.2 *biodegradable volatile solids, n*—The organic matter fraction; the biodegradable portion of total solids that volatilizes to carbon dioxide and other gasses when a compost or feedstock is combusted at 550°C in the presence of excess air, % g g^{-1}.

3.3 *fixed solids, n*—The inorganic matter, or mineral residue of total solids that remains when a compost or feedstock is combusted at 550°C in the presence of excess air; equivalent to *ash*, % g g^{-1}.

3.4 *moisture content, n*—The liquid fraction (percentage) of a compost or feedstock that evaporates at 70±5°C, % g g^{-1}.

3.5 *total solids, n*—The solid fraction (percentage) of a compost or feedstock that does not evaporate at 70±5°C, which consists of fixed solids, biodegradable volatile solids, and volatile solids that are not readily biodegradable, % g g^{-1}.

3.6 *volatile solids, n*—The sum of biodegradable materials, non-biodegradable materials, and biodegradable materials that do not degrade during the retention time allowed for composting, that volatilize to carbon dioxide and other gasses when a compost or feedstock is combusted at 550°C in the presence of excess air, % g g^{-1}.

4. Summary of Test Methods

4.1 *Method 03.02-A Unmilled Material Ignited at 550°C without Inerts Removal*—Quick-Test to determine moisture and total solids content at 70±5°C, and total ash and volatile solids content by combustion at 550°C in the presence of excess air and reported on an oven dried basis of an unsieved, as-received finished or in-process compost or feedstock sample.

4.1.1 This test is recommended for samples where no consideration need be given to inert materials and biodegradable materials that do not degrade during the retention time allowed for composting.

4.2 *Method 03.02-B Milled Material Ignited at 550°C with Inerts Removal*—Analytical test to determine biodegradable volatile solids contents (organic matter) by combustion at 550°C in the presence of excess air and reported on an oven-dried basis from an air-dried (36°C), sieved and milled compost sample from which non-biodegradable or biodegradable materials that do not readily humify have been removed.

4.2.1 This test method provides an estimate of biodegradable volatile solids. Compensation for inert materials and biodegradable materials that do not degrade during the retention time for composting prior to combustion is accomplished by their removal through sample preparation prior to performing this test.

4.3 *Method 03.02-C Unmilled Material Ignited at 550°C with Inerts Removal*—A test to determine moisture and total solids content at 70±5°C, biodegradable volatile solids content (organic matter) by combustion at 550°C in the presence of excess air and reported on an oven-dried basis of a sieved, as-received finished or in-process compost, or feedstock sample.

4.3.1 The test employs a calculated weighting method to compensate for inert materials and for biodegradable materials that do not degrade during the retention time allowed for composing.

5. Significance and Use

5.1 Carbon dioxide and other gasses are evolved when the biodegradable volatile solids portion (organic matter) of material is combusted at 550°C. The ash residue is mineral fraction of compost that remains in the fixed solids, or inorganic content of total solids.

5.2 Biodegradable volatile solids (organic matter) and ash content in feedstock and compost are two of three material categories in total solids, or dry matter. Total solids, or dry matter include:

5.2.1 Organic Matter (OM), which is occasionally referred to as Biodegradable Volatile Solids (BVS);

5.2.2 Ash or fixed solids (Ash, inorganic matter, or minerals); and

5.2.3 Volatile Solids that are not readily biodegradable (VS).

6. Interference and Limitations

6.1 Biodegradable volatile solids (organic matter) is determined on a compost sample that is sieved and whose inert contaminants are removed, i.e., during inert classification. Samples must be thoroughly blended and properly split (subdivided) prior to drying, milling and inert removal. Unmilled coarse samples are almost always more variable than finely milled samples. A small aliquot of milled material will more closely resemble the bulk milled sample than will a small aliquot of unmilled coarse material.

6.1.1 The presence of materials that are not readily biodegradable such as wood chips and man-made inerts such as plastics cause over estimation of the sample biodegradable volatile solids.

6.1.2 The presence of man-made inerts such as metal and glass cause over estimation of the sample ash content.

6.2 Volatile residues may accumulate on glass surfaces when ashing temperatures are too low (<500°C) and/or the duration of the ashing process is too short. If volatile residues accumulate on the ashing vessel, the volatile solids determination will be low.

6.3 A sample is oven dried at 70±5°C rather than 105°C, to minimize volatile loss of carbon compounds during determinations of total solids. The significance of this error increases with increasingly mature materials where the relative volatile solids measure diminishes to less than ten percent the total solids.

6.4 Negative errors in volatile solids can be produced through the loss of volatile matter from samples that require prolonged drying at relatively high temperatures. This error may become significant with feedstock where total solids is very high relative to volatile solids. If this condition exists, consider measuring for quantities of suspect volatile components by another test, such as the total organic carbon test (Method 04.01).

7. Sample Handling

7.1 *Method 03.02-A Unmilled Material Ignited at 550°C without Inerts Removal*—Perform this test on a feedstocks, in-process and finished composts. The material may contain unclassified inert material. This

test should be implemented as the standard for the Volatile Solids Reduction test (Test Method 05.10-A).

7.1.1 *Compost Samples*—This test is best performed in conjunction with the total solids and moisture test. Use the same sample for volatile solids determination (50 cm^3 aliquot of prepared material).

7.1.2 *Feedstocks Samples*—Increase sample size to 400 cm^3 for feedstock sample analysis. This test is best performed in conjunction with the total solids moisture test. Use the same sample for volatile solids determination (400 cm^3 aliquot of prepared material).

7.2 *Method 03.02-B Milled Material Ignited at 550°C with Inerts Removal*—Perform this test with a 250 cm^3 aliquot of material screened through a 9.5-mm sieve, air-dried at 36°C, with man-made inerts >2 mm and materials >2 mm not readily biodegradable removed from the sieved material, and milled to a fine powder texture.

7.2.1 Material for this test should conform to the marketing specifications established for compost product distribution.

7.3 *Method 03.02-C Unmilled Material Ignited at 550°C with Inerts Removal*—Perform this test on 50 cm^3 aliquot of material screened through a 9.5 mm sieve, oven-dried at 70±5°C, with man-made inerts and materials not readily biodegradable removed from the sieved material.

7.3.1 Material for this test should conform to the marketing specifications established for compost product distribution.

Test Method: Ash. Unmilled Material Ignited at 550°C without Inerts Removal						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	03.02-A	03.02-A		03.02-A		03.02-A		

03.02-A UNMILLED MATERIAL IGNITED AT 550°C WITHOUT INERTS REMOVAL

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Apparatus for Method A

8.1 *Balance*—analytical, with accuracy of ±0.001 g.

8.2 *Furnace*—forced air muffle, set at 550°C.

8.3 *Evaporation Dish*—Pyrex beaker, use 150-mL beaker with compost samples, and 500-mL beakers with feedstocks.

8.4 *Watch Glass*—2.5 cm (2 in.) diameter for compost, or 5 cm (4 in.) diameter for feedstock.

8.5 *Desiccator Cabinet*—vacuum with desiccant tray containing a color indicator of moisture concentration or an instrument indicator.

9. Reagents and Materials for Method A

9.1 *None required.*

10. Procedure for Method A

10.1 *Preparation of Evaporating Dish:*

10.1.1 Heat a clean beaker or crucible to 105°C for 0.5 h to 1.0 h to drive off all hygroscopic moisture.

10.1.2 Place heated beaker in desiccator cabinet to keep it dry, and allow to cool to approximately 27°C.

10.1.3 Record tare weight immediately prior to use.

10.2 *Determine Initial Sample Weight:*

10.2.1 Place sample beaker in forced-air oven set at 70±5°C for approximately 18 h – 24 h, until weight change and moisture loss diminishes to nil.

10.2.2 Transfer 50 cm^3 of oven-dried compost to the 150-mL beaker. For feedstocks, transfer approximately 400 cm^3 of oven-dried material to a 500-mL beaker.

10.2.2.1 Disregard the mass of inerts when using this method.

10.2.3 Weigh and record gross weight of beaker and sample, subtract beaker weight from gross weight to determine net weight of the oven-dried sample (dw).

10.3 *Ash Weight:*

10.3.1 Place the watch glass, concave side facing up, on top of the beaker and transfer it to the forced-air muffle furnace; slowly ramp furnace temperature to 550°C, ash at 550°C for 2 h, and then slowly ramp furnace temperature to approximately 200°C.

10.3.2 Transfer beakers containing ashed samples to a desiccator and cool to approximately 27°C.

10.3.3 Weigh and record gross weight of beaker and sample (AshW).

10.4 *Calculations*—determine ash content (fixed solids) and volatile solids content.

11. Calculations for Method A

11.1 *Calculate Total Solids and Moisture content as percentages as-received wet weight basis:*

$$TS = (dw \div ARW) \times 100 \qquad \textit{Equation 11.1.1}$$

$$M = [1 - (dw \div ARW)] \times 100 \qquad \textit{Equation 11.1.2}$$

where:

TS = total solids, % g g^{-1},
M = percent moisture, % g g^{-1},
dw = sample net oven-dried weight determined at 70±5°C, ±0.001 g, and
ARW = sample net weight at as-received moisture, ±0.001 g.

11.2 *Calculate organic matter (OM) and ash (Ash) content as percentages of total solids on an oven dry weight basis:*

$$Ash = (AshW \div dw) \times 100 \qquad \textit{Equation 11.2.1}$$

$$VS = [1 - (AshW \div dw)] \times 100 \qquad \textit{Equation 11.2.2}$$

where:

Ash = percentage of solids at 550°C, % g g^{-1},
VS = percentage of solids volatilized at 550°C, % g g^{-1},
dw = net oven-dry weight at 70±5°C, ±0.001 g, and
AshW = net ash weight at 550°C, ±0.001 g.

COMMENT—As compost becomes increasingly humified (biological degradation), the relative content of biodegradable volatile solids approaches zero.

Test Method: Ash. Milled Material Ignited at 550°C with Inerts Removal						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
				03.02-B		03.02-B		

03.02-B MILLED MATERIAL IGNITED AT 550°C WITH INERTS REMOVAL

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

12. Apparatus for Method B

12.1 *Analytical Balance,* with of ±0.001 g accuracy.

12.2 *Crucibles*—high-form ceramic with cover, 20-mL.

12.3 *Desiccator Cabinet*—vacuum with desiccant tray containing color indicator of moisture concentration or an instrument indicator.

12.4 *Forced-Air Drying Oven,* for operation at 70±5°C.

12.5 *Muffle Furnace*—forced-air for operation at 550°C.

13. Reagents and Materials for Method B

13.1 *None required.*

14. Procedure for Method B

14.1 *Preparation of Evaporating Crucible:*

14.1.1 Heat a crucible to 105°C for 0.5-1.0 h to drive off all hygroscopic moisture.

14.1.2 Place heated crucible in desiccator cabinet to keep it dry, and allow to cool to approximately 27°C.

14.1.3 Record tare weight immediately prior to use, ±0.001 g.

14.2 *Sample Aliquot*—Transfer 10 cm^3 aliquot of prepared, (air-dried), material to a dry, tared crucible.

14.3 *Initial Weight*—Weigh and record gross weight of crucible and sample, subtract crucible weight from gross to determine net air-dried weight (inert-free sample would be air-dried at 36°C).

14.4 *Determine Oven Dry Weight (dw):*

14.4.1 Place sample crucible in forced-air oven set at 70±5°C for approximately 18 h – 24 h, until weight change and moisture loss diminishes to nil.

14.4.2 Place sample crucible in desiccator and cool to approximately 27°C.

14.4.3 Weigh and record gross weight of crucible and sample.

14.4.4 Subtract crucible weight from gross to determine net oven-dried weight (dw) of sample.

14.5 *Determine Ash Weight:*

14.5.1 Place the capped crucible to the forced air muffle furnace; slowly ramp furnace temperature to 550°C, ash at 550°C for 2 h, and then slowly ramp furnace temperature to approximately 200°C.

14.5.2 Place the crucible and ashed sample in a desiccator and cool to approximately 27°C.

14.5.3 Weigh and record gross weight of crucible and sample.

14.5.4 Subtract crucible weight from gross to determine net ash weight.

14.6 Perform Calculations.

15. Calculations for Method B

15.1 *Calculate organic matter (OM) and ash (Ash) content as percentages of total solids on an oven dry weight basis:*

$$Ash = (AshW \div dw) \times 100 \qquad \textit{Equation 15.1.1}$$

$$OM = [1 - (AshW \div dw)] \times 100 \qquad \textit{Equation 15.1.2}$$

where:

Ash = percentage of fixed solids remaining after combustion at 550°C, % g g^{-1},

BVS = the organic matter fraction of solids, percentage of readily biodegradable solids volatilized at 550°C, % g g^{-1},

dw = oven-dry weight of the test sample aliquot, g

OM = BVS = LOI organic matter, and

AshW = net ash weight at 550°C, g.

Test Method: Ash. Unmilled Material Ignited at 550°C with Inerts Removal							**Units:** % g g^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								03.02-C

03.02-C UNMILLED MATERIAL IGNITED AT 550°C WITH INERTS REMOVAL

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

16. Apparatus for Method C

16.1 *Evaporating Dish*—Pyrex beaker of 150-mL capacity.

16.2 *Watch Glass*—Pyrex, 5 cm (2 in.) diameter.

16.3 *Forced-Air Drying Oven,* for operation at 70±5°C.

16.4 *Muffle Furnace,* for operation at 550°C.

16.5 *Desiccator Cabinet*—With a desiccant containing a color indicator of moisture concentration or an instrument indicator.

16.6 *Sieve*—#5 mesh (4 mm).

16.7 *Analytical Balance*—capable of weighing to 100 g, with ±0.1 mg accuracy.

17. Reagents and Materials for Method C

17.1 *None required.*

18. Procedure for Method C

18.1 *Preparation of Evaporating Dish:*

18.1.1 Heat a clean beaker to 105°C for 0.5 h - 1.0 h to drive off all hygroscopic moisture.

18.1.2 Place heated beaker in desiccator cabinet to keep it dry, and allow to cool to approximately 27°C.

18.1.3 Record tare weight of dry beaker immediately prior to use.

18.2 *Preparation of Sample:*

18.2.1 Transfer 50 cm^3 of as received sample material as received to a tared Pyrex beaker. Obtain and record total as-received weight of sample in beaker, ±0.001 g.

18.2.2 Place beaker with sample into forced-air drying oven set at 36°C and dry for 24 h - 48 h.

18.2.3 Remove beaker and cool in desiccator for minimum of 1 h. Record dry weight of beaker contents.

18.2.4 Empty the air-dried sample onto a #5 sieve (4-mm) and remove stones and manufactured inert material, such as metal fragments, glass shards, sharps, leather, textiles, hard and film plastics, and all material that will not biologically degrade, or oversized biodegradable material such as wood chips and twigs that will not degrade during the retention time of the composting process.

18.2.4.1 Using a soft spatula, scrape the remaining material across the sieve, and collect the sieve accepts (4 mm and under).

18.2.4.2 Oven dry the hand-sorted trash at 70±5°C for 18 h - 24 h until sample weight change due to moisture loss diminishes to nil. Weigh and record the mass of inert material removed (T_R) from the air-dry >4-mm sample.

18.2.5 Recombine the sieve accepts (under 4 mm) and oversized (over 4 mm) biodegradable material. Weigh and record weight of recombined compost sample.

18.2.6 Place the beaker with recombined compost in a forced-air drying oven set at 70±5°C and dry for 18-24 h until sample weight change due to moisture loss diminishes to nil.

18.2.7 Remove the beaker and cool in a desiccator for a minimum of 1 h. Determine oven-dried weight of beaker contents, (dw).

18.3 *Sample Analysis:*

18.3.1 Transfer the recombined oven-dried sample with inert material removed into a tared 150-mL tared Pyrex beaker.

18.3.1.1 Weigh and record gross weight of sample and beaker. Determine compost sample net weight.

18.3.2 Place the watch glass, concave side facing up, on top of the beaker and transfer it to the forced-air muffle furnace; slowly ramp furnace temperature to 550°C, ash at 550°C for 2 h, and then slowly ramp furnace temperature to approximately 200°C.

18.3.2.1 Transfer beakers containing ashed samples to a desiccator and cool to approximately 27°C.

18.3.2.2 Weigh and record gross weight of the ashed sample plus beaker. Determine sample net weight, (AshW).

18.3.3 Sieve the beaker contents through a #20 mesh (4-mm sieve) with a wire brush; weigh the over #20 mesh material and record net weight of any small trash fragments that escaped removal in step 18.2.4. This trash consists of stones and other inerts not volatilized at 550ºC, (T_A).

19. Calculation for Method C

19.1 Calculate ash content as a percentage of total solids, i.e., dry matter on an oven dry weight basis:

$$Ash = [AshW - T_A] \div [dw + T_R] \times 100 \quad \textit{Equation 19.1}$$

where:

Ash = fixed solids of biodegradable fraction remaining after combustion at 550°C, % g g^{-1},

AshW = net ash weight including fine trash (T_A), combusted at 550°C, g,

dw = net oven-dried weight of recombined sample at 70±5°C, g,

T_A = net weight of trash remaining after ashing, over #20 mesh materials, g, and

T_R = net oven-dried weight of trash removed prior to ashing, hand-sorted and removed, g.

19.2 Calculate the organic matter (OM, biodegradable volatile solids) as a percentage of total solids, i.e., dry matter on an oven dry weight basis:

$$OM = [100 - Ash] \quad \textit{Equation 19.2}$$

where:

OM = organic matter fraction, biodegradable volatile solids evolved at 550°C, % g g^{-1}, and

Ash = fixed solids remaining after combustion at 550°C, from Equation 19.1, % g g^{-1}.

03.02 METHODS SUMMARY

20. Report

20.1 *Report the Following Information:*

20.1.1 Express results for Organic Matter (OM, %) and Biodegradable Volatile Solids (BVS, %) as a percentage for the ratio, mass of volatilized compost per mass of oven-dried compost, % g g^{-1}.

20.1.2 Express results for percent ash (Ash, %) as a percentage for the ratio, unit mass of ash per unit mass of compost on an oven-dried basis, % g g^{-1}.

20.1.3 Report test method number.

20.2 *Minimum Detection Limit*—Record unit mass to an accuracy of ±0.005 g.

21. Precision and Bias

21.1 *Percent Ash and Biodegradable Volatile Solids:*

21.1.1 *Test Method 03.02-A Unmilled Material Ignited at 550°C without Inerts Removal:*

21.1.1.1 *Feedstocks, In-Process Materials, Finished Compost* —The precision and bias of these tests have not been determined. Data are being sought for use in developing a precision and bias statement.

21.1.2 *Method 03.02-B Milled Material Ignited at 550°C with Inerts Removal*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

21.1.3 *Method 03.02-C Unmilled Material Ignited at 550°C with Inerts Removal*—The precision of this test were determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

21.1.3.1 Precision was determined using 10 subsamples taken from one field composite sample for each of three locations and two months (1993).

Table 03.02-C1 Percent Ash, %. Variability is expressed as percent relative standard deviation, % CV.

Median	*Std Dev*	*% CV*	*Number of Samples*
54.0	3.36	6.3	10
82.5	1.35	1.6	10
59.4	4.10	6.9	10
38.6	1.45	3.7	10
53.4	0.77	1.5	10
40.6	4.64	11.9	10

Table 03.02-C2 Biodegradable Volatile Solids, %. Variability is expressed as percent relative standard deviation, CV.

Median	*Std Dev*	*% CV*	*Number of Samples*
46.0	3.36	7.2	10
17.5	1.35	7.7	10
40.6	4.10	10.1	10
61.4	1.45	2.4	10
46.6	0.77	1.6	10
59.4	4.64	7.6	10

NOTE 1C—Coefficient of Variation, %CV = Standard Deviation ÷ Mean × 100.

22. Keywords

22.1 ash; biodegradable volatile solids; fixed solids; moisture content; total solids; volatile solids

Test Method: Bulk Density. *Refer to* Method 03.01 Air Capacity for method protocols						**Units:** kg m^{-3} dw (Lb yd^{-3})		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	03.01-A	03.01-A	03.01-A	03.01-A				
		03.01-B		03.01-B		03.01-B		
								03.01-C

03.03 BULK DENSITY

REFER TO METHOD 03.01 AIR CAPACITY FOR METHOD PROTOCOLS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the measurement of bulk density for composted materials.

1.1.1 Refer to TMECC 03.01-A or 03.01-B, or TMECC 03.01-C for protocols.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

ASTM D 2980-71, Standard Test Method for Volume Weights, Water Holding Capacity, and Air Capacity of Water-Saturated Peat Materials. *In* Annual Book of ASTM Standards, Vol. Vol. 04.08. (Re-approved 1990).

Haug, Roger T., The Practical Handbook of Compost Engineering, Lewis Publishers, 1993.

Water holding Capacity, Volume Mass and Air Capacity of Water-Saturated Peat. ASTM D 2989-71. p 77. *In* Peat Testing Manual. National Research Council of Canada, Technical Memorandum No. 125. 1979.

Laboratory Procedure for the Preparation of Solid Waste and Related Materials for Analysis. p 3. *In* Methods of Solid Waste Testing. 1973. US EPA. Office of Research and Monitoring. US EPA-6700-73-01, Part I.

3. Terminology

3.1 *bulk density, n*—Weight per unit volume of compost, calculated and reported on an oven dry weight basis, 70±5°C, kg m^{-3}.

3.2 *equivalency, n*—At STP, 1 g of deionized water ≡ 1 cm^3 of deionized water ≡ 1 mL of deionized water ≡ 1 mL of air space ≡ 1 cm^3 of air space.

4. Summary of Test Methods

4.1 *Method 03.03-A (See Method 03.01-A Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Unsieved Compost)*—A compost sample taken from the pile, as is, of known volume and mass is systematically transferred to a graduated beaker and bulk density is determined.

4.2 *Method 03.03-B (See Method 03.01-B Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Sieved Compost)*—A compost sample taken from the pile, as is, of known volume, is sieved and systematically transferred to a graduated beaker and bulk density is determined.

4.3 *Method 03.03-C (See Method 03.01-C Field Density, Free Air Space and Water-Holding Capacity)*—A composite compost sample collected from a pile or windrow is systematically transferred to a bucket in the field and weighed to estimate bulk density.

4.4 *Method 03.10-D (See Bulk Density and Water-Holding Capacity, of Water-Saturated Compost, Modified ASTM D 2980-71)*—A composite compost sample is systematically transferred to a burette and weighed to estimate bulk density, dw basis.

4.4.1 Volume weights, porosity/pore space, water holding capacity, bulk density, compost volumes and air volume are determined on both weight and volume basis from these data.

4.4.2 The bulk density test result is reported as kg m^{-3} with the weight expressed on a 70±5°C dry weight basis, where volume is measured after the sample is systematically packed as indicated. A dry weight adjustment ratio is determined on a separate parallel aliquot of compost material.

5. Significance and Use

5.1 Bulk density is weight per unit volume of compost.

5.1.1 Bulk density based on as-received moisture per unit volume at as-received moisture can be used to estimate transportation requirements.

5.1.2 Bulk density is used for conversion from application rate in mass per unit area to thickness of application layer (e.g., tons per acre to inches).

5.2 As compost matures, its organic carbon content decreases due to chemical and biological conversions of organic carbon to carbon dioxide. As this occurs, structural support provided by various carbon compounds degrades and collapses, causing the remaining inorganic materials (salts and metals) to compact. At the molecular level, this compaction of structure is referred to as molecular close-packing. This process concentrates materials, both biodegradable and non-biodegradable. The result is a higher unit of mass per unit volume in aged, composted material than that of the original feedstock blends.

03.03 METHODS SUMMARY

6. Report

6.1 *Report the Following Information*—Express results for bulk density as the ratio of mass per unit volume of compost on an oven-dried basis (70±5°C) to the nearest ±1.0 kg m^{-3} (Lb yd^{-3}) dw basis.

6.1.1 Report moisture content of compost sample aliquot used to determine bulk density. Describe sample condition.

7. Precision and Bias

7.1 *Bulk Density:*

7.1.1 The precision and bias of the tests for Methods 03.03-A, B, and C have not been determined. Data are being sought for use in developing a precision and bias statement.

7.1.2 *Method 03.10-D Bulk Density, and Water-Holding Capacity of Compost Material, Modified ASTM D 2980-71*—The precision of this test were determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

7.1.2.1 Precision was determined using 10 subsamples taken from a field composite sample for each of three sites for two sampling periods, (1993).

Table 03.03-1 Bulk Density, kg m^{-3}. Precision estimates for < 6.4 mm as-received MSW compost material (1993).

Median	*Std Dev*	*% CV*	*Number of Samples*
400	5.0	1.3	10
390	5.0	1.3	10
380	6.7	19.5	6
410	6.0	1.6	10
350	9.0	2.7	10
340	1.5	4.4	10

NOTE 1—Coefficient of Variation, %CV = Standard Deviation ÷ Mean × 100.

8. Keywords

8.1 bulk density; maturity; weight; volume

Test Method: Wettability. Two Methods.							**Units:** see methods	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		03.04-A		03.04-A	03.04-A	03.04-A		03.04-A
		03.04-B		03.04-B	03.04-B	03.04-B		03.04-B

03.04 WETTABILITY

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers proposed tests covers to measure wettability characteristics of compost.

1.1.1 *Method 03.04-A—Wicking Rate of Compost.*

1.1.2 *Method 03.04-B—Water-Drop Penetration Rate.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 02.02-B Sample Sieving for Aggregate Size.

Method 02.02-C Man-made inert Removal.

Method 02.02-D Milling and Grinding Samples, Harrison.

Method 02.02-E Milling and Grinding Samples, Munter.

Method 03.09-A Total Solids and Moisture.

2.2 *Scientific Literature:*

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3. Terminology

3.1 *absorb, v*—To take (something) in through pores or interstices.

3.2 *absorption, n*—A process in which one material (the absorbant) takes in or absorbs another (the absorbate); as the absorption of moisture by compost.

3.3 *adsorption, n*—The accumulation of gases, liquids, or solutes on the surface of a solid or liquid.

3.4 *critical micelle concentration (CMC)*—The concentration of a surfactant above which it predominantly forms aggregates including micelles in solution. Addition of more surfactant results in the formation of more aggregates while leaving the monomer concentration more or less unchanged.

3.5 *fines, n*—Particles with a diameter < 2 mm.

3.6 *hydrophilic, adj*—Having an affinity for water; readily absorbing or dissolving in water. A hydrophilic surface is a surface that is readily wet by water e.g., clean glass.

3.7 *hydrophobic, adj*—The propensity of a solid to repel water. Repelling, tending not to combine with, or incapable of dissolving in water. A hydrophobic surface is a surface that is not readily wet by water e.g., Teflon.

3.8 *micelle, n*—A spherical assembly that forms spontaneously in aqueous solution. The hydrophilic heads of the surfactants are exposed to the water with the hydrophobic tails forming the solid core.

3.9 *surface tension, n*—A property of liquids arising from unbalanced molecular cohesive forces at or near the surface. In an effort to minimize its surface tension or energy the surface area of the liquid at the liquid-air interface tends to contract. The surface tension of a liquid is commonly reported as mN m^{-1} and the surface tension of water is 73 mN m^{-1}.

3.10 *surfactant, n*—A surface active agent that reduces the surface tension of fluids that coat a material. The molecules contain a section that is hydrophobic (a hydrocarbon tail) and a section that is hydrophilic ± the headgroup). Examples include:

3.10.1 *Brij 58, n*—A non-ionic surfactant, chemical name: polyoxyethylene (20) cetyl alcohol p-t-octyl phenol. The molecular weight is 1120 and the critical micelle concentration is 0.008 g 100 mL^{-1} or 71.4 μ*M*.

3.10.2 *Nonidet P-40, n*—A non-ionic surfactant. Chemical name: polyoxyethylene (9) p-t-octyl phenol. The molecular weight is 603 and the critical micelle concentration is 0.017 g 100 mL^{-1} or 282 μ*M*.

3.10.3 *Tween 20, n*—A non-ionic surfactant, chemical name: polyoxyethylene (20) sorbitol monostearate. The molecular weight is 1230 and the critical micelle concentration is 0.006 g 100 mL^{-1} or 9.92 μ*M*.

3.11 *wettability, n*—the propensity of a surface to adsorb moisture. The state or condition of being wettable, or the relative affinity of liquid for the surface of a solid, such as the affinity of water for paper or leather. Wettability increases directly with increasing affinity, as measured by the contact angle formed between the liquid and the solid. This increases from non-wettability at an angle greater than 90 degrees to complete wettability when the contact angle is 0 degrees.

4. Summary of Test Methods

4.1 *Method 03.04-A Wicking Rate of Compost*—The wettability threshold is determined by establishing whether a sample of compost can re-wet to more than 35 percent moisture by capillarity. A series of prepared compost samples with varying moisture contents are exposed to water. The rate and amount of water absorption by each sample is measured. The wettability threshold is reached when the time required to rewet the compost sample exceeds a predetermined maximum allowable time period.

4.1.1 As-received compost is passed through a 4-mm sieve, subdivided into 50 cm^3 samples and placed in a forced air oven preheated to 36°C. Individual samples are sequentially removed during drying, weighed and the percent moisture content calculated. The same sample is then transferred to a filtered beaker. The filtered beaker is sealed onto the top of a second glass flask using ground glass joints. Water, from a modified burette fitted with a stop-cock, flows into the flask through an inlet tube at the bottom of the lower flask. Flexible tubing connects the delivery end (bottom) of the burette with the lower flask's inlet tube. The receiving end (top) of the burette is sealed with a rubber stopper fitted with a narrow glass capillary tube (tube open to air). The capillary tube is positioned to ensure a constant head of pressure.

4.1.2 The amount of water absorbed by the compost is determined by monitoring the rate and amount of water that exits the burette and passes to the sample (Fig 03.04-A1). At the end of a run, the filtering device containing the sample is re-weighed and placed in an oven to determine moisture content of moistened compost sample. The run is repeated for each 50 cm^3 compost sample, each with different moisture content (i.e., 0, 5, 10, 15, 20, 25 and 30%).

4.1.3 *Wettability Threshold*—The lowest percent moisture (wet weight basis) that compost may attain without limiting its ability to re-absorb moisture is determined If the kinetics of wetting is too slow, additional runs are conducted with non-ionic surfactants dissolved in water at or below the critical micelle concentration.

4.2 *Method 03.04-B Water-Drop Penetration Rate*—As-received compost is passed through a 4-mm sieve, subdivided into 20 cm^3 samples and placed in a forced air oven preheated to 36°C. Individual samples are removed from the oven at periodic intervals during drying, weighed and the initial percent moisture content of the sample is calculated. Approximately six cm^3 of compost is placed into three petri dishes and the sample surface is flattened.

4.2.1 Three drops (100 µL) of water are carefully placed onto each flattened compost sample. The time (up to 1 h) required for the nine drops to penetrate the compost is visually monitored and recorded. Runs are repeated with compost samples at varying moisture contents, (e.g., 0, 5, 10, 15, 20, 25 and 30%).

4.2.2 The average time required for droplets to penetrate compost is reported for each compost moisture content.

5. Significance and Use

5.1 Three practical applications for determining the compost wettability threshold and re-wetting index are presented: in-process and moisture management during compost curing; in-plant dust control; and efforts to decrease shipping costs without diminishing compost quality. Each case illustrates how changes in the wettability properties of a compost occur during drying and how they impact management decisions and costs. In practice, wettability measurements as described in this section are rarely used by compost operators during composting or when planning compost uses. The methods most likely to be used for technical accuracy are provided. However, rapid, onsite assessments by compost site operators will often be the first choice approach because of cost and time limitations.

5.1.1 M*aintaining optimum moisture content during the curing phase of compost*—Biological degradation consumes moisture during curing. The rate of degradation diminishes to nil when the compost moisture content falls below a minimum biological activity moisture threshold of approximately 40%. Curing composts are regularly monitored for moisture content and require frequent additions of water and further blending to maintain moisture conditions above the minimum threshold. In some cases, curing compost is mistakenly left to dry below its wettability threshold and as a result, cannot be rapidly re-moistened. Approved surfactants are sometimes used to accelerate the re-moistening process of dry compost; ionic particles (e.g. clays) that readily rewet are sometimes blended into a dried compost to hasten re-wetting.

5.1.2 *Dust Control*—When compost moisture falls below the dust threshold (~35%), fines and other small particles become airborne with minimal agitation. This dust is a nuisance and a potential worker health hazard. Often, water is used to control dust, however, rapid drying often induces radical changes in the surface structure of compost fines that can significantly alter the absorptive characteristic of compost, making it difficult to rewet air-dried fines and small particles.

5.1.3 *Reduce Shipping Costs*—Compost products are shipped from composting facility to market, as either bulk or bagged material. The bulk mass for shipping may be reduced more than 50% by air-drying finished material prior to shipment; this reduces the cost of shipping. The end-use and method used to dry the compost prior to shipping dictate the re-moistening requirements of compost. For applications on farms, rewetting is usually unnecessary, because mixing with moist soil upon application provides adequate moisture. It would also be unnecessary to rewet compost used to manufacture blends of potting mixtures that will also contain ionic fines (e.g. clays).

6. Literature Review

6.1 *Wetting of Solids, Review of Basic Principles:*

6.1.1 *Introduction*—The ability of water to wet depends upon the surface characteristics of the material. If the surface contains a significant fraction of hydrophilic groups (-SO_4Na, -COOK, -COOH, -OH, R_3-NH, -Si(OH-) surrounded by layers of water molecules, the surface is readily wet by water. In contrast, if the surface contains a significant fraction of hydrophobic groups (e.g. hydrocarbons, carbon fluoride, -CH-, -CH_2- CH_3 CF), the surface is not readily wet by water. Feedstocks used to produce the finished compost significantly influence the surface characteristics of compost. For example, as the plastics content of feedstock increases, the ability of the resulting compost to absorb and retain moisture decreases, because water will not wet most stable plastics (e.g., carbon fluoride containing plastics, Teflon). In comparison, animal manure composts are generally hydrophilic and more readily wet by water.

6.1.2 *Surface characteristics* of feedstocks and compost sometimes change upon drying. As an example, if compost dries during the curing process or during storage, some of the dried fines will readily accept moisture, while others will require wetting agents or surfactants before they may rapidly remoisten to a desired level. If significant changes occur in the surface chemistry of compost (i.e., change from hydrophilic to more hydrophobic groups), it will be less able to re-absorb moisture. Changes in surface chemistry vary with drying temperature, drying rate, and relative moisture content of dry fines. Use of surfactants can be avoided if significant chemical changes do not occur and the moisture content of compost is maintained above a minimum wettability threshold level.

6.1.3 *Definition*—The technical definition for wetting of a solid is that the contact angle is zero or so close to zero that the liquid spreads over the solid easily. Non-wetting implies that the angle is greater than 90° and liquid tends to ball up and run off the surface. The definition of contact angle and

illustrations of non-wetting to wetting solids are shown in Figure 03.04-1.

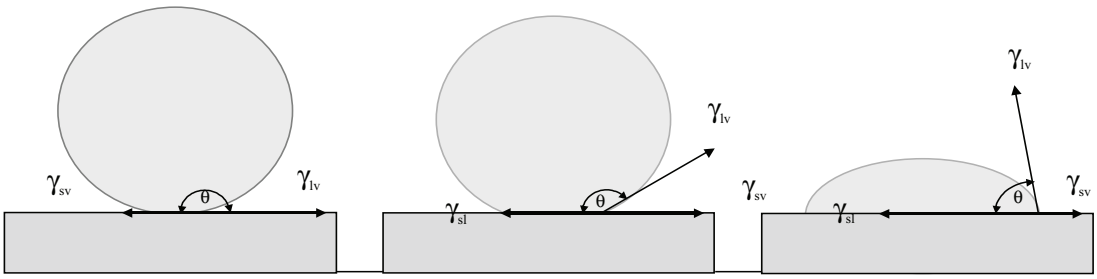

Fig 03.04-1 Definition of a contact angle on a non-porous surface.

6.1.3.1 Consider what happens when a spherical droplet of water is placed on a non-porous solid in contact with water vapor. The droplet will either spread (wet) or ball-up (not wet) on the solid surface. At equilibrium, there is no more change in the area of the solid covered and the surface tensions or energies of the various interfaces are balanced. This is expressed by Young's equation:

$\gamma^{sv}-\gamma^{sl} = [\gamma^{lv}\cos\theta]$ *Equation 6.1.3.1*

where:

γ^{sv} = surface tension at the solid-vapor interface,

γ^{sl} = surface tension at the solid-liquid interface,

γ^{lv} = surface tension at the liquid-vapor interface, and

θ = contact angle

6.1.3.2 If the droplet completely wets the surface, (i.e., the contact angle is zero), then Young's equation is not valid and the spreading coefficient (S^{slv}) is used to describe the imbalance of the energies:

$S^{slv} = [\gamma^{sv}-(\gamma^{sl}+\gamma^{lv})]$ *Equation 6.1.3.2*

6.1.3.3 To encourage spreading (S^{slv} is positive) γ^{sl} and γ^{lv} should be as small as possible. In practice this is accomplished by adding to the liquid phase a surfactant that adsorbs at both the solid-liquid and liquid-vapor interfaces, and lowers the interfacial tensions.

6.1.4 *Surface Tension*—Water has a surface tension of 72.8 mN m^{-1} and does not readily wet surfaces of low surface energy; examples of such surfaces are graphite, paraffin and many plastics. It does wet polar surfaces; contact angles of 0° are measured on clean glass, clean quartz, uncontaminated gold (Adamson, 1982) and freshly cleaved mica. In contrast, ethanol is a liquid with a low surface tension (22 mN m^{-1}) and forms droplets with very small contact angles (< 10°) on most surfaces. There are a few plastics that it does not wet. Teflon or polytetrafluroethylene (PTFE) is one of the materials that ethanol does not readily wet, it forms droplets with a contact angle of about 40° (Hu and Adamson, 1977). As a general rule, a liquid only wet solids that are of higher surface energy than its surface tension.

6.1.5 Surface tension of most liquids decreases linearly with increasing temperature— The surface tension of water decreases 0.138 mN m^{-1} per °C (Kayser, et al., 1976) the surface tension of ethanol decreases 0.086 mN m^{-1} per °C (Adamson, 1982). Common aqueous electrolytes like NaCl increase the surface tension of water. The surface tension of 1 M NaCl solution is about 74 mN m^{-1} (Adamson 1982), while nonpolar solvents like ethanol decrease the surface tension of water.

6.2 *Wetting a Porous Medium*

6.2.1 *C*ompost is a porous material and wetting involves not only the spreading on the surface, but the penetration of liquid into the pores by capillarity (Fig 03.04-2).

6.2.2 In this case the wetting process is related to capillary rise where the driving force for wetting is the pressure difference across the curved surface of a meniscus (ΔP) and for a spherical meniscus of radius, r, this can be described with Laplace's equation:

$\Delta P = [2\gamma^{lv}\cos\theta \div r]$ *Equation 6.2.2*

6.2.3 For a finite contact angle $0° < \theta < 180°$ the pressure gradient can be written as:

$\Delta P = [2(\gamma^{sv}-\gamma^{sl}) \div r]$ *Equation 6.2.3*

6.2.4 To improve penetration it is necessary to make γ^{sl} as small as possible, except for a perfectly wetting liquid, ($\theta = 0$) where it is necessary to make γ^{lv} large because equation 6.2.1 becomes:

$\Delta P = [2\gamma^{lv} \div r]$ *Equation 6.2.4*

6.3 The net goal to improve penetration for a perfectly wetting liquid is to add a wetting agent or surfactant that reduces γ^{sl} without at the same time reducing γ^{lv}. It is unlikely that water will act as a perfectly wetting liquid for compost and the objective in practice is usually to add a surfactant that makes γ^{sl} as small as possible.

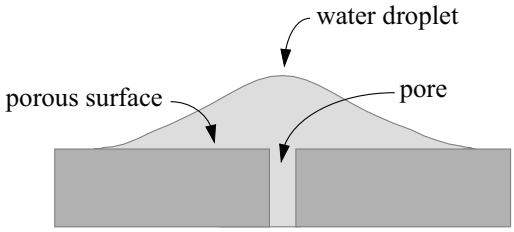

Fig 03.04-2 Water droplet on a porous surface. Water both spreads across surface and is absorbed through pores.

6.3.1 *Surfactants*—Non-ionic (e.g., Tween 20) as opposed to ionic surfactants are more suitable to use for compost, because ionic surfactants tend to be more reactive and have the potential to affect biological activity. For example, they are better denaturing agents (denature proteins and membranes) and can reduce microbial activity. The amount of surfactant to add is usually small and always at or below the critical micelle concentration, adding it at higher concentrations will not lower the surface tension of water and will only increase the chance of toxicity.

6.3.2 *Heterogeneous media*—Finished compost is a heterogeneous media with varying surface structure and porosity. Even within a sample there is variability with respect to porosity and surface structure. Relationships presented above are useful for understanding the principles behind the wettability of compost or the water repellency characteristics of compost, but cannot be rigorously applied to heterogeneous material.

6.4 *Summary of Potentially Adaptable Methods*—The current methods used to measure the water repellency of soils and other porous media like pharmaceutical powders and fabrics are reviewed.

6.4.1 *Water-Drop Penetration Rate*—This is a method proposed by Watson and Letey (1970) to measure the repellency of water on soil. A water droplet ~ 50 µL is placed on the surface of a soil core or soil sample and the time taken for the drop to completely penetrate the soil is measured. The average penetration time of ten drops is reported for each sample. There are several variations of this test. Measurement of penetration times for entry times of up to 1 h (Berglund, *et al.,* 1996). Measure penetration times for entry times of up to 4 min (King 1981) and classification of the soils depending upon the time the water takes to enter the soil (Dekker and Ritsema, 1994). Ten classes were distinguished: (i) wettable (<5 s); (ii) slightly water repellent (5 to 60 s); (iii) strongly water repellent (60 to 600 s); (iv) severely water repellent (600 s to 1 hr) and extremely water repellent with six classes based on the time needed for infiltration (v) 1-2 h (vi) 2-3 h (vii) 3-4 h (viii) 4 to 5 h (ix) 5 to 6 and (x) > 6 h.

6.4.1.1 This is a quick test that could be adapted for use with compost. It is not quantitative and not suitable for determining the wettability threshold, but it would at least indicate if compost were readily rewettable. It could also be used to test surfactants added to improve the kinetics of wetting.

6.4.2 *The Molarity of Ethanol Test*—This method was also suggested by Watson and Letey (1970) and is another common method adapted and used by several groups to assess the repellency of soil (King, 1981; Berglund and Persson, 1996). There are several forms of this method. Drops (50 µL) of aqueous ethanol solutions of concentrations from 0 to 7 M at 0.2 *M* intervals are placed on soil samples and their entry time is recorded. The degree of repellence is represented by the molarity of ethanol that penetrated the surface in 10 seconds.

6.4.2.1 Ethanol is a solvent with a low surface tension that readily wets most surfaces. The higher the concentration of ethanol in aqueous solution the lower the surface tension of the liquid and the more likely it will wet low energy (and generally hydrophobic) solids. Therefore the more hydrophobic the surface groups of compost the higher the concentration of ethanol required for wetting and the more water repellent the compost. Unlike soil, some compost may contain inert plastic components that are not readily wettable, even by low surface tension liquids like ethanol. The presence of plastics could bias results, so methods that require the use of ethanol may not be appropriate for compost.

6.4.3 *Determining the liquid-soil contact angle in soil and sand by capillary rise*—This method was outlined by Emerson and Bond (1962) and Letey *et al.* (1961) and was used by King (1981) and others (Hammond and Yuan, 1968). It uses the Young-Laplace equation to determine the contact angle. From Equation 3.3.1 the pressure difference across the meniscus must be balanced by the hydrostatic pressure drop in the column of liquid. $\Delta P = h\Delta\rho g$ where: h: maximum height of capillary rise, g: acceleration due to gravity; $\Delta\rho$ difference between density of water and air. Historically the capillary rise method was used to determine the surface tension of liquids and is very accurate provided the liquid wets the glass capillary and the exact solutions of the Young-Laplace equation are used.

6.4.3.1 If it can be assumed that the porous media is equivalent to a bundle of capillaries of average radius r, and the moisture content below the maximum height of capillary rise is constant, equation 6.4.3.1 can be used to determine an apparent contact angle in porous media. Because there are two unknowns θ and r, the

approach is to compare the capillary rise of two identically packed columns; immerse one column in a low surface tension liquid (e.g. ethanol) and assume the liquid completely wets the surface (cos(θ) =1) and immerse the other in water and determine the angle at the soil-water interface from:

$\cos\theta = [\Delta P \div \Delta P_o] \times [\gamma^{lve} \div \gamma^{lvw}]$ *Equation 6.4.3.1*

$\cos\theta = [hg\Delta\rho \div h_o g\Delta\rho_o] \times [\gamma^{lve} \div \gamma^{lvw}]$ *Equation 6.4.3.2*

where:

ΔP = pressure drop across the air-water meniscus,
γ^{lvw} = surface tension at the water-air interface,
$\Delta\rho$ = difference in density between water and air,
h = maximum height of capillary rise of water,
θ = contact angle at the soil-water interface,
ΔP_o = pressure drop across the ethanol-air meniscus,
γ^{lve} = surface tension at the ethanol-air interface;
$\Delta\rho_o$ = difference in density between ethanol and air, and
h_o = maximum height of capillary rise of ethanol.

6.4.3.2 This approach was first implemented by Letey *et al*., (1962) with sand columns. They presented evidence that ethanol penetrated sand with the same rate, independent of the treatment whereas the rate of penetration of water depended on treatment. They then concluded that ethanol wets sand (contact angle = 0°) and used it to determine a contact angle at the sand-water interface. As mentioned, this is probably a reasonable assumption, ethanol is a liquid with a low surface tension (22 mN m^{-1}) and forms droplets with very small contact angles (< 10°) on many surfaces.

6.4.4 *Capillary Rise*—There are two forms of this test determining the equilibrium and dynamic capillary rise:

6.4.4.1 *Equilibrium capillary rise*—Glass tubes were made hydrophobic by treating them with paraffin dissolved in xylene, they were then filled with sand and immersed in alcohol or water. The capillary rise was monitored periodically and measured after 24 h (Letey *et al*. 1962). This time was assumed to be sufficient for equilibration.

6.4.4.2 *Dynamic capillary rise*—Emerson and Bond (1962) developed this technique for soils. This method requires only about 15 minutes. A positive head is used to push the liquids through a small diameter glass or plastic tube filled with soil or sand. The tube was immersed in a large water or ethanol reservoir of known height and the average position of the wetting front was measured every 15 sec for about 15 to 30 min. The rate of change of the wetting front dx/dt was plotted against the inverse of the wetting front. The line is extrapolated to zero to determine the height of wetting front when dx/dt =0. After subtracting the positive head the maximum capillary rise obtained in water (h) or ethanol (h_o) is determined and the contact angle at the soil-water interface is calculated with equation 6.3.3.2.

6.4.4.3 There are two reasons that make this method unsuitable for assessing the wettability threshold. The capillary rise would be difficult to measure in wet (up to 30% moisture) compost and the capillary rise of water in compost has to be compared with that measured in ethanol. In the method proposed water is absorbed through compost by capillary forces, but the height of the capillary rise is not measured only the rate that compost absorbs water.

6.4.5 *Dynamic Contact Angle Measurement*—The most commonly used method for measuring the contact angle of powders is to measure the height of a large drop on a pre-saturated porous substrate. The substrate is compressed into a tablet and a drop applied to the tablet is not drawn into the material because the pores are already saturated. In practice this measurement is difficult, the surface has to be flat and it is not easy to prevent a water film forming on the surface of the tablet. Another method for determining the wettability of a porous material is to monitor the change in the contact angle with time (Link and Schlunder, 1996). This is similar to the water drop penetration time, but more systematic because the shape of the contact angle is measured at time intervals. A tablet is formed by compressing the powder particles to < 100 μm radius and kept isothermal by placing the tablet on a heating plate. A droplet (3 μL) is discharged from a syringe with the aid of a micrometer screw. The wetting process is observed and recorded by a video camera. The contact angle of the drop decreases from 180° to some static value after time t. If the contact angle dropped from 180° (spherical drop) to zero very quickly, the substrate would be wet. The rate at which the droplet disappears indicates the kinetics of wetting. Penetration increases with increased porosity and temperature.

6.4.5.1 This method could be adapted for use with compost, the rate at which the droplet disappears and the final size of the contact angle would indicate the propensity for compost to wet. Unfortunately, this method requires fairly expensive equipment, specialty software, and a highly trained technician. In addition to the standard laboratory equipment, a camera, commercial grade VCR, computer interface, computer and image software are required. The technician would need to be able to identify interface boundaries which would be very difficult with rough porous surfaces.

6.4.6 *Infiltration Methods*—These methods involve packing a column with soil or sand and determining the rate at which the water flows through the medium.

Columns are positioned horizontally, vertically upward, or vertically downward (Letey, *et al*., 1962; King, 1981; and Berglund and Persson, 1996).

6.4.7 *Comparison of Test Methods*—King (1981) compared tests used to assess the water repellence of soils. He examined over 100 sandy soils and compared the molarity of the ethanol test, dynamic contact angle measurement, water drop penetration time and an infiltration method that used gravity to assist flow. He found strong correlation among tests. The soils were categorized according to results of the test. However, reproducible tests were only observed with air and oven-dried samples. Increasing the moisture content of the soils affected the reproducibility of the tests, in all cases it was recommended that tests be performed with air- or oven-dried soil (King, 1981). His observations implied that repeatability might become limiting for tests performed on composts with a moisture content above 0 % (wet weight basis).

7. Interference and Limitations

7.1 Interference and limitations of this test are not determined. Data are being sought for use in developing an interference and limitations statement.

8. Sample Handling

8.1 Perform this test on feedstocks or finished composts. The material may contain levels of unclassified inert material that meets end-use standards.

8.1.1 Procedures are performed on a sieved sample aliquot of compost maturity classes of interest, including feedstock if appropriate.

8.1.2 This test may be performed in conjunction with sample sieving as outlined under Method 02.02-B and Method 02.02-C.

Test Method: Wettability. Wicking Rate of Compost							**Units:** % at $t_{critical}$	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		03.04-A		03.04-A	03.04-A	03.04-A		03.04-A

03.04-A WICKING RATE OF COMPOST

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTED BY—Dr. Brenda F. Farrell, Edaphos International

9. Apparatus for Method A

9.1 *Drying Oven*—forced air, vented, temperature set at 36°C and 70±5°C.

9.2 *Bag*—20 brown paper, #6, or equivalent container suitable for air-drying samples.

9.3 *Sieve*—4-mm mesh, plastic or stainless steel.

9.4 *Analytical Balance*—accurate to ±0.001 g.

9.5 *Wicking Apparatus*—filtering device (modified beaker with a sintered glass bottom), flask, burette, capillary tube, rubber stopper and tubing. Refer to Fig 03.04-A1.

9.6 *Syringe with tuberculin needle*—10 of 10-mL syringe fitted with a 5-cm tuberculin needle.

9.7 *Volumetric flasks*—three, 500-mL.

10. Reagents and Materials for Method A

10.1 *Water*—deionized 17 MΩ·cm water; surface tension of the water used in the experiments should be measured and reported with the results.

10.2 *Brij 58*—71.4 μ*M* aqueous solution. Add 250 mL of water to a 500-mL volumetric flask. Add 0.04 g of Brij 58. Fill to a final volume of 500 mL with water. (optional)

10.3 *Nonidet P-40*—282 μ*M* aqueous solution. Add 250 mL of water to a 500-mL volumetric flask. Add 0.085 g of Nonidet P-40. Fill to a final volume of 500 mL with water. (optional).

10.4 *Nitric acid, 10%*—Add 750 mL of water into a 1-L volumetric flask; add 100 mL of concentrated nitric acid. Fill to a final volume of 1 L with water.

CAUTION—The nitric acid solution is highly corrosive and must be made up in fume hood.

10.5 *Tween 20™*—9.92 μ*M* aqueous solution. Place 250 mL of water to a 500-mL volumetric flask. Add 0.0065 g of Tween 20™. Add more water to a final volume of 500 mL. (optional).

10.6 *Other Surfactants*—Use other suitable non-ionic surfactant made up at its critical micelle concentration.

11. Apparatus Preparation for Method A

11.1 Acid wash all glass components (filter device, flask and burette) with 10% nitric (NO_3) acid and rinse thoroughly with distilled water.

11.2 Soak all components in ethanol for 1 h and rinsed with water (17 MΩ·cm resistivity).

11.3 Allow all glass components to dry.

11.4 Assemble apparatus as illustrated in Fig 03.04-A1.

11.4.1 Fill the flask and burette with water to a level in line with the lower side of the sintered glass.

11.4.2 Close the stopcock and fill the burette to the top with water.

11.4.3 Cap the burette with a soft rubber stopper coated with glycerol that is fitted with the capillary tube.

11.4.4 Position the capillary tube to ensure that its lower end is directly in line with the lowest measured increment on the burette and directly in line with the lower side of the sintered glass.

11.4.5 When the stopper is secure, remove excess water with the syringe fitted with a tuberculin needle. Insert the needle through the rubber stopper and use the syringe to extract excess water from the burette. Sufficient water is removed to bring the water meniscus into alignment with the upper-most volume mark of the burette, and simultaneously, air reaches the very bottom end of the capillary tube. Remove the needle and the perforation in the rubber formed by the needle will close and seal itself providing the rubber stopper is malleable and properly lubricated.

12. Sample Preparation for Method A

12.1 Pass approximately 750 cm^3 of compost through a 4-mm sieve to provide at least 600 cm^3 of < 4 mm sieved material.

12.2 Blend and thoroughly mix sieved compost.

12.3 Subdivide the < 4 mm material into separate 50 cm^3 samples, weigh and place each sub sample into a forced-air oven preheated to 36°C. Take three separate aliquots of the excess sieved material and determine their average moisture content (as-received compost moisture, at 70±5°C; or consider oven-drying at 105°C if rapid analysis is needed.

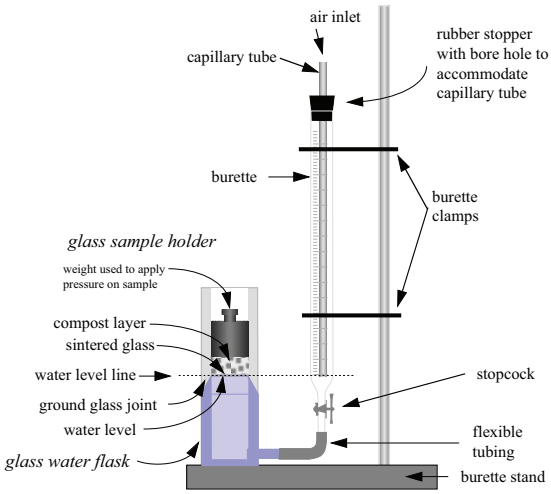

Fig 03.04-A1 Wicking rate of compost apparatus assembly

NOTE—Samples are run in pairs, removed from the oven at regular time intervals (e.g., after 1, 2, 4, 8 24 and 36 h), cooled to room temperature and individually subjected to the wicking rate test. As mentioned, runs of the test are repeated at time intervals.

12.4 Determine moisture content of partially dried compost by oven drying at 70±5°C overnight.

13. Procedure for Method A

13.1 Place 45-50 cm^3 of partially dried compost into each of two filtering devices (modified beaker with a sintered glass bottom) and weigh.

13.2 Press compost sample into a flat slab (height of approximately 1 cm) at constant force (1-10 N, ~1 kg; the required mass will vary with bulk density of the compost) with a weight of equal diameter to that of the filtered sample flask (refer to Fig 03.04-A1 for illustration). This ensures that the compost sample is in intimate contact with the sintered glass.

13.3 *Start Run*—Place the filtering device that is filled with compost on top of the flask and open the stopcock of the burette. This is the start of the experiment, where t=0. If the compost moisture content is above the wettability threshold, the sample absorbs water; the water meniscus of the burette will move down and a corresponding volume of air will enter the burette via the capillary tube.

13.4 Measure and record the height of the meniscus every 15 sec for 15 min.

13.5 Thereafter, measure the height of the meniscus every 15 min for up to 3 h, or up to the maximum allowable re-wetting time as dictated by the compost re-wetting operation.

13.6 Calculate the mass of water absorbed by the compost for each time interval and plot mass of water against time, (assume that 1 mL $H_2O \equiv 1$ g H_2O).

13.6.1 *Validation Step*—At the end of the run, weigh the filtering device and determine the change in weight of the compost due to the absorption of water.

13.6.2 Place filtering device in oven at 70±5°C overnight and determine the % moisture of the compost (wet weight basis).

13.7 If the samples pass this test (re-wet to ~35% moisture), repeat steps 13.1 through 13.6 with each sample pair as they are removed from the drying oven, where an acceptable series of moisture contents would be 0, 5, 10, 20, 25 and 30% moisture. If not, stop the test and proceed to Step 13.9 to evaluate surfactants to enhance the wetting characteristics of water.

NOTE A2—If the final % moisture content of the compost is greater than 35% the compost is considered to be wet. This implies that the initial state of the compost was at or above the wettability threshold. If the final % moisture content is less than 35% the compost is not considered to be wet. This implies that the initial state of the compost was below the wettability threshold.

13.8 *Determine the wettability threshold of compost*—This is the minimum percent moisture (wet weight basis) that compost may attain without affecting its ability to re-absorb moisture. If the percent moisture content of compost is greater than 35% the compost is considered wet. For example, if the initial moisture content is 0% and the compost can be wet to 35% moisture at a sufficiently fast rate then 0% moisture is the wettability threshold. The rate at which compost re-wets also defines the wettability and this will depend upon the problem at hand and/or the end use of the compost. Report the time taken to reach 35% moisture.

13.9 *Surfactants*—Enhance the degree and rate of wetting using a modification of the Method 03.04-B Water-Drop Penetration Rate by adding non-ionic surfactants to water.

13.9.1 Follow the procedures outlined for Method 03.04-B.

13.9.2 Return to Step 13.1, but use a water solution containing the wetting agent in place of pure water.

13.9.3 Report type and concentration of surfactant used to improve the rate and degree of wetting.

14. Calculations and Corrections for Method A

14.1 Determine % moisture of as received compost:

$M = 1 - (ODW \div ARW) \times 100$ *Equation 14.1*

14.2 Determine % moisture of partially dried compost:

$A = 1 - (B \div C) \times 100$ *Equation 14.2*

14.3 Determine % moisture of compost after wetting:

$D = 1 - (E \div F) \times 100$ *Equation 14.3*

14.4 Compare % moisture of compost after wetting with compost of 35% moisture. if ΔDF is greater or equal to -2 then the compost is wet:

$\Delta DF = G - 35$ *Equation 14.4*

14.5 Determine rate of wetting:

$H = [IR - FR] - [CF \times (IR - FR)]$ *Equation 14.5*

where:

ODW = oven dried weight of compost, g,
ARW = as received weight of compost, g,
M = percent moisture of as received compost, %,
B = oven dry weight of compost that was dried for a period of time, g,
A = percent moisture of partially dried compost, %,
C = weight of compost after drying it for a period of time, g,
D = percent moisture of re-wetted compost, %
E = oven dry weight of compost in filtering device after wetting, g,
F = weight of compost in filtering device before wetting, g,
H = volume of water absorbed by compost, mL,
ΔDF = difference between percent moisture of rewet compost and compost of 35%,
IR = initial burette reading, mL,
FR = final burette reading, mL, and
CF = correction factor, volume of capillary per mL of the water in burette, mL mL^{-1}.

14.6 Plot mass of water adsorbed against time assuming 1 mL of water is equivalent to 1 g of water.

14.7 Determine wettability threshold of compost. For the hypothetical data set example shown in Table 03.04-A1, the wettability threshold is 15%. Refer to Table 03.04-A1 for further explanation.

15. Interpretation of Method A

15.1 *Re-wetting Dried Materials*—If a sample below its wettability threshold must be rewet, a surfactant at a concentration below its CMC is used to rewet the sample. The test is repeated using a surfactant solution to assure that it may be used to re-wet the compost in question at the desired rate.

15.2 *Monitoring Compost Moisture*—During the composting process, the moisture status of the compost must be maintained above the wettability threshold to avoid dust problems and to maintain adequate moisture for maintaining biological activity. Table 03.04-A1 illustrates a hypothetical scenario where the wettability threshold was identified to be 15%, given the maximum allowable time for re-wetting (t = critical).

Table 03.04-A1 Hypothetical data set indicating wettability threshold of a compost sample given the maximum allowable time for re-wetting.

Moisture Content of Compost Sample (%)	*Final Moisture of Compost (%)*	*Time to Rewet is less than t=critical*
30	35	yes
25	34	yes
20	36	yes
15	34	yes
10	28	No
5	25	No
0	20	No

Test Method: Wettability. Water-drop penetration Rate							**Units:** min $\%^{-1}$	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		03.04-B		03.04-B	03.04-B	03.04-B		03.04-B

03.04-B WATER-DROP PENETRATION RATE

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTED BY—Dr. Brenda F. Farrell, Edaphos International

16. Apparatus for Method B

16.1 *Drying Oven*—forced air, vented, temperature set at 36°C.

16.2 *Bag*—brown paper, # 6, or other drying container.

16.3 *Sieve*—4-mm mesh, plastic or stainless steel.

16.4 *Hamilton Syringe*—250-µL.

16.5 *Petri Dishes*—20, glass dim: 5 x 1 cm.

17. Reagents and Materials for Method B

17.1 *Water*—type II, deionized 17 MΩ·cm water, minimum standard.

17.2 *Brij 58*—71.4 µ*M* aqueous solution. Place 250 mL of water to a 500-mL volumetric flask. Add 0.04 g of Brij 58. Add more water to a final volume of 500 mL. (optional)

17.3 *Nonidet P-40*—282 µ*M* aqueous solution. Place 250 mL of water to a 500-mL volumetric flask. Add 0.085 g of Nonidet P-40. Add more water to a final volume of 500 mL. (optional).

17.4 *Tween 20*—9.92 µ*M* aqueous solution. Place 250 mL of water to a 500-mL volumetric flask. Add 0.0065 g of Tween 20. Add more water to a final volume of 500 mL. (optional).

17.5 *Other Surfactants*—Use other suitable non-ionic surfactant made up at its critical micelle concentration.

18. Sample Preparation for Method B

18.1 Pass approximately 200 cm^3 of compost through a 4-mm sieve to provide at least 175 cm^3 of sieved material.

18.2 Blend sieved compost together

18.3 Subdivide the < 4 mm material into subsamples. Place each 20 cm^3 subsample into a bag. Take three small subsamples, each from a different sample, to determine the average moisture content of the as-received compost (oven dried at 70±5°C for 24 h or at 105°C for 1 h or until change in mass is nil).

18.4 Place bags containing 20 cm^3 samples in forced air oven preheated to 36°C.

18.5 At regular time intervals (e.g. after 0, 1, 2, 4, 8 24 and 36 h), sequentially remove each 20 cm^3 sample from the oven and subject it to the water-drop penetration test.

19. Procedure for Method B

19.1 Place 6 cm^3 of partially dried compost into a set of three petri dishes and gently flatten the compost slightly with a weighted flat surface.

19.2 Place 3 drops (100 µL volume) of water on each compost sample. Make sure the drops are at least 1 cm apart.

19.2.1 *Surfactant Solutions*—if a dry sample fails to rewet and if the goal is to rewet this material,

19.2.1.1 In addition to water drops place 10 (100 µL) drops of surfactant solution to the surface of the compost. The concentration of the surfactant should be at or below the critical micelle concentration.

19.2.1.2 Compare the time required for water droplets to infiltrate the flattened sample (average of ten drops) and the time required for the wetting agent to penetrate the surface of compost.

19.2.1.3 Establish which of the surfactants (Tween 20, Brij 58, Nonidet P-40) increases the rate of wetting.

19.2.1.4 Repeat runs outlined below using lower dilutions of surfactant to identify the minimum effective surfactant concentration.

19.3 Observe and Record the time required for all water drops to penetrate the flattened compost sample.

19.4 Stop the run at 1 h.

19.5 Determine the average time for penetration, if time is greater than 1 h, simply report penetration time to be greater than 1 h.

NOTE 1B—this experiment should be conducted in a room where the humidity and temperature are controlled and or constant (i.e., air-conditioned).

19.6 Repeat steps 19.1 to 19.5 for pairs of compost sub-samples as they are removed from the drying oven and allowed to cool to room temperature.

20. Calculations and Corrections for Method B

20.1 Determine the average observed time required to complete penetration for each pair of samples for each sample moisture tested:

$$t_{avg} = [t_1 + t_2 + ... + t_n] \div n \qquad \textit{Equation 20.1}$$

where:

t_{avg} = average time to penetrate compost, min, and

t = time it takes each water drop to penetrate compost, min,

n = run number, (i.e., 1 through 9).

03.04 METHODS SUMMARY

21. Report

21.1 *Method 03.04-A Wicking Rate of Compost*—Report wettability threshold of compost, % at $t_{critical}$, where % is moisture content and $t_{critical}$ is the maximum allowable time for rewetting; actual time required to rewet compost sample; bulk density of compost; force added to compress compost (kg); concentration and type of surfactant used to improve wetting, if required; temperature and humidity in room where experiments were conducted; and surface tension of water and surfactant solutions.

21.2 *Method 03.04-B Water-Drop Penetration Rate*—Report average time required for water drop to penetrate compost for each moisture level tested; initial moisture content of compost; bulk density of compost; surface tension of water and surfactant solutions; and temperature and humidity in room during experiments.

22. Precision and Bias

22.1 *Method 03.04-A Wicking Rate of Compost*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

22.2 *Method 03.04-A Water-Drop Penetration Rate*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

23. Keywords

23.1 absorption; adsorption; sorption; fines; hydrophobic; hydrophilic; surface tension; surfactant; wettability; wettability index

Test Method: Film Plastics. One Method.							**Units:** $cm^2\ m^{-3}$	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
					03.05-A			03.05-A

03.05 FILM PLASTICS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

COMMENT—This test was devised for use with mixed municipal solid waste and lawn and garden composts. The outlined methods conform to conventional image scanning and processing protocols. Refer to your digital image processing software users' manual for use instructions.

NOTE—Test methods presented in this section of TMECC should not be used in place of ASTM methods to certify degradability of plastics or other degradable materials (e.g., ASTM D6400-99: *Standard Specification for Compostable Plastics*).

1. Scope

1.1 This section covers the quantification of film plastics in air-dried composted materials.

1.1.1 *Method 03.05-A Film Plastic Surface Area Determinations Using Digital Processing.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

NIH Image 1.61. Public Domain Image Processing Software for Macintosh™. US National Institute of Health. http://www.nist.gov/lispix/imlab/labs.html. August 2, 1993.

3. Terminology

3.1 *plastic, n*—Any of various organic compounds produced by polymerization, capable of being molded, extruded, cast into various shapes and films, or drawn into filaments. Plastic objects are undesirable contaminants in finished composts.

3.2 *film plastic, n*—Shredded sheet plastic, man-made inert. The weight of film plastic is insignificant relative to the bulk weight of as-received compost.

4. Summary of Test Method

4.1 *Method 03.05-A Film Plastic Surface Area Determinations Using Digital Processing*—A proposed method using digital scanning and image processing to quantify the relative surface area of film plastic present per unit volume of compost.

5. Significance and Use

5.1 Relative surface area determination of film plastics, measured in $cm^2\ m^{-3}$, can indicate its potential impact under different compost use scenarios. The total mass of film plastics relative to bulk compost is very small. Error associated with mass determinations throughout the process of sieving and drying may mask the aesthetic significance of the presence of film plastics. Also, organic carbon determinations with CO_2 detection may be inflated due to the presence of petroleum-based carbon in film plastics.

6. Interference and Limitations

6.1 *Method 03.05-A Film Plastic Surface Area Determinations Using Digital Processing*—Image processing software must be properly calibrated to avoid inaccurate estimates of film plastics surface areas.

6.2 Data are being sought for further development of a Interference and Limitations statement.

7. Sample Handling

7.1 *Method 03.05-A Film Plastic Surface Area Determinations Using Digital Processing*—Material used in this test should represent in-process compost product at 45-60% moisture (wet weight basis). The sample aliquot should be unsieved, as-received.

7.1.1 *Quantity*—250 cm^3, four replicates, finished compost.

Test Method: Film Plastics. Surface Area Determinations Using Digital Processing							**Units:** $cm^2\ m^{-3}$	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
					03.05-A			03.05-A

03.05-A FILM PLASTIC SURFACE AREA DETERMINATIONS USING DIGITAL PROCESSING

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTED BY—Wayne H. Thompson, Edaphos International

8. Apparatus for Method A

8.1 *Sieve*—4-mm, plastic, nylon or other durable mesh.

8.2 *Desk Top Computer*—with image scanning and processing capabilities.

8.3 *Scanner*—digital, flatbed, 8-bit gray scale capable (minimum).

8.4 *Bottle and Cap*—wide-mouth, 500-mL or other appropriate container with cover.

8.5 *Paper*—opaque, A4 or 8.5×11 in. sheet.

8.6 *Acetate Sheet*—clear, A4 or 8.5×11 in. sheet.

8.7 *Area Calibration Standard*—flat disc of known area dimension to be scanned as area calibration reference with film plastic fragments.

8.8 *Image Processing and Analysis*—software (e.g., *Image-J* - Image Processing and Analysis in Java. http://rsb.info.nih.gov/ij/).

9. Reagents and Materials for Method A

9.1 *Coating Agent*—Graphite powder, or other suitable translucent material to coat transparent film plastics.

CAUTION—Graphite powder spills on or near electronic equipment can create severe complications and cause equipment failures.

10. Procedure for Method A

10.1 *Air Dry Sample*—Analytically transfer a 1000 cm^3 sample aliquot to a paper bag or other permeable container and air dry for at least two days at 36°C until weight change diminishes to nil.

10.2 Press the 250 cm^3 air dried sample aliquot through a 4-mm sieve.

NOTE 1A—Determine cumulative surface area of film plastic fragments > 4 mm. A 4-mm sieve will allow the flexible 4-mm film plastic sections to pass through a sieve without detection.

10.3 Inspect the material remaining on the screen and manually separate film plastics.

10.4 Determine the surface area of film plastic.

10.4.1 Coat transparent film plastic. Transfer film plastics into wide-mouth 500-mL bottle. Add approximately 50 cm^3 of opaque coating agent. Cap bottle. Shake vigorously until all transparent film plastics are coated. Separate excess coating agent from coated film plastics using a sieve.

10.4.2 Assemble samples and calibration standard for scanning. Transfer the coated film plastic to a sheet of clean, white paper. Arrange the coated film plastics. Do not overlap fragments of film plastics. Cover the film plastic and paper with clear acetate sheet.

NOTE 2A—Include a sample of known dimension for image calibration.

10.4.3 Scan assembled sample as an 8-bit gray-scale image. Place the prepared sample sheet face-down onto flatbed scanner. Capture the image as directed by the scanning software instructions. Save the scanned image to a file for further processing.

10.4.4 Determine surface area of each assembled sample. Using image processing software, import the captured images of film plastics and convert the image from gray scale to binary (i.e., 0 = black = plastics, 255 = white = background).

10.4.4.1 Determine area in pixels for each piece of film plastic on white paper sheet.

10.4.4.2 Determine area in pixels for sample with known surface area.

10.4.4.3 Sum area in pixels for all fragments of film plastic on the sheet. Multiply the sum by the area of one pixel, cm^2.

10.5 Repeat steps 10.4.4.1 through 10.4.4.3 three times for each sheet of prepared samples until technician proficiency improves and variability between runs diminishes to nil.

10.6 Repeat step 10.2 through step 10.4 for each of the four 250 cm^3 subsample of compost.

11. Calculations for Method A

11.1 *Determine Area Conversion Coefficient:*

$$C = \sum(P_{Sn} \div A_S) \div n \qquad \textit{Equation 11.1}$$

where:

C = conversion factor used to convert pixels to cm^2, cm^2 per pixel,

P_S = number of pixels occupied by sample with known area,

A_S = area cm^2 of sample with known area, and

n = replication number (minimum = 3).

11.2 *Convert Film Plastic Area Measures from Pixels to Area Units, cm^2:*

$$A_{FP} = P_{FP} \times C \qquad \textit{Equation 11.2}$$

where:

C = conversion coefficient to convert from pixels to cm^2, cm^2 per pixel, from equation 11.1,

P_{FP} = number of pixels occupied by graphite coated film plastic, and

A_{FP} = area of graphite coated film plastic, cm^3.

11.3 *Calculate total surface area for 1000 cm^3 sample:*

$$T_{FP} = \sum(A_{FP}) \qquad \textit{Equation 11.3}$$

where:

A_{FP} = surface area of film plastic for individual 250 cm3 samples, and

T_{FP} = total surface area of film plastic in 1,000 cm^3 sample

11.4 *Correct Area to Volume Measurement (from 1000 cm^3 sample volume to 1.0 m^3):*

$$A_T = T_{FP} \times 1{,}000 \qquad \textit{Equation 11.4}$$

where:

A_T = total area of film plastics per cubic meter of compost, $cm^2\ m^{-3}$,

T_{FP} = total surface area of film plastic in 1,000 cm^3 sample, and

1000 = conversion factor for cm^3 to m^3.

03.05 METHODS SUMMARY

12. Report

12.1 Film plastic surface area is reported in square centimeters (cm^2) per cubic meter (m^3) of as-received compost, $cm^2\ m^{-3}$. Report the sample mean and standard deviation when three or more samples from the same bulk are submitted for analysis.

13. Precision and Bias

13.1 *Method 03.05-A Film Plastic Surface Area Determinations Using Digital Processing*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

14. Keywords

14.1 inerts; plastic; film plastics; image processing; digital imagery; surface area; volume

Test Method: Glass Shards, Metal Fragments and Hard Plastics. One Method.						**Units:** % g g^{-1} compost dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
						03.06-A		03.06-A

03.06 GLASS SHARDS, METAL FRAGMENTS AND HARD PLASTICS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

NOTE—Test methods presented in this section of TMECC should not be used in place of ASTM methods to certify degradability of plastics or other degradable materials (e.g., ASTM D6400-99: *Standard Specification for Compostable Plastics*).

1. Scope

1.1 This section covers the quantification of glass shards, metal fragments and hard plastics in finished compost.

1.1.1 *Method 03.06-A Glass Shards, Metal Fragments and Hard Plastics Wet Sieving Technique*.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

ASTM D 2217-85, Standard Practice for Wet Preparation of Soil Samples for Particle-Size Analysis and Determination of Soil Constants. *In* Annual Book of ASTM Standards, Vol. 04.08. (Re-approved 1993).

3. Terminology

3.1 *glass, n*—Any of a large class of materials with highly variable mechanical and optical properties that solidify from the molten state without crystallization, are typically made by silicates fusing with boric oxide, aluminum oxide, or phosphorus pentoxide, are generally hard, brittle, and transparent or translucent, and are considered to be supercooled liquids rather than true solids.

3.2 *metal, n*—Any of a category of electropositive elements that usually have a shiny surface, are generally good conductors of heat and electricity, and can be melted or fused, hammered into thin sheets, or drawn into wires. Typical metals form salts with nonmetals, basic oxides with oxygen, and alloys with one another. An alloy of two or more metallic elements. An object made of metal.

3.3 *plastics, n*—Any of various organic compounds produced by polymerization, capable of being molded, extruded, cast into various shapes and films, or drawn into filaments used as textile fibers. Objects made of plastic.

3.4 *sieve, n*—A utensil of wire mesh or closely perforated metal, used for straining or sifting compost.

3.5 *wet sieving, v*—To wash compost and/or inert materials through a sieve with water and to collect those materials that do not pass through the sieve for further classification.

4. Summary of Test Methods

4.1 *Method 03.06-A Glass Shards, Metal Fragments and Hard Plastics Wet Sieving Technique*—A destructive wet sieve technique for rapid quantification of foreign matter in finished compost.

4.1.1 A proposed method using a wet sieve technique is described. Replicated aliquots of as-received compost are wet sieved to isolate glass shards, metal fragments and hard plastics. The presence of foreign matter relative to a bulk sample of compost material is determined and reported as a percentage on dw basis.

5. Significance and Use

5.1 Glass shards (4-mm to 13-mm) can pose a human and animal hazard with unprotected exposure or through direct ingestion. Metal fragments can pose the same hazard, and could be a potential source of trace elements upon interaction with soil. Hard plastic can be an aesthetic concern and in large quantities may affect physical properties of a compost-amended soil, (e.g., soil coloring, heat retention, drainage).

6. Interference and Limitations

6.1 Interference and limitations for this method have not been determined. Information is being sought to develop a Interference and Limitations statement.

7. Sample Handling

7.1 *Sample Size*—1000 cm^3, four replicates at 250 cm^3 each.

7.2 *Sample Preparation*—as-received finished compost.

Test Method: Glass Shards, Metal Fragments and Hard Plastics. Wet Sieve Technique						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
						03.06-A		03.06-A

03.06-A WET SIEVING TECHNIQUE

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

NOTE—This test should not be used in place of ASTM methods to certify degradability of plastics or other degradable materials, i.e., ASTM D6400-99: *Standard Specification for Compostable Plastics*.

COMMENT—This proposed test was prepared by W.H. Thompson for use with mixed solid waste compost.

8. Apparatus for Method A

8.1 *Water Delivery System*—2-cm ($^3/_4$ in.) rubber, tubing, garden hose or other equivalent .

8.2 *Evaporation Dish*—four 250-mL beakers, four 100-mL beakers, glass.

8.3 *Sieve*—4-mm mesh, 20-cm diameter, plastic or equivalent.

8.4 *Tweezers*—sized for extracting ~ 4 mm fragments from sample.

8.5 *Forced Air Drying Oven*—vented, capable of maintaining constant temperature, 70±5°C.

8.6 *Analytical Balance*—capable of weighing 100 g, accurate to ±0.001 g.

9. Reagents and Materials for Method A

9.1 *Water*—continuous flow source, tap water.

10. Procedure for Method A

10.1 Transfer four 250 cm^3 aliquots of unsieved finished compost to each of four tared 250-mL beakers.

10.2 Determine and record weight of each 250 cm^3 as-received moist sample aliquot.

10.3 Determine and record the sample total solids and moisture content on a parallel aliquot.

10.4 Wash each replicate sample through a 4-mm sieve.

10.5 Inspect sieve accepts, wet material remaining on the screen, and separate clearly identifiable inert materials.

10.5.1 *Man-Made Inerts*—glass shards, metal fragments and hard plastics,

10.5.2 *Natural Inorganic Inerts*—stones and concretions, and

10.5.3 *Organic Fragments*—organic materials that are not readily biodegradable such as wood chips, fragments of paper, etc.

10.6 Transfer the recovered and classified inert materials to clean, tared beakers.

10.7 Oven dry recovered inerts at 70±5°C for 18 h to 24 h, until weight change or moisture loss diminishes to nil.

10.8 Separate and classify oven-dried inert materials into descriptive categories, (e.g., glass shards, metal fragments, and hard plastics).

10.9 Determine and record the individual net weight for each class of inerts, ±0.001 g.

10.10 Repeat steps 10.4 through 10.9 for each replicate, minimum of three replicates, four replicates are recommended.

10.11 Perform calculations for each class of inert.

11. Calculations for Method A

11.1 Perform the following calculations for each replicate. Determine the mean and standard deviation for each inert class.

11.1.1 *Inert Content by Type, % w/w:*

$$X_i = A \div B \qquad \textit{Equation 11.1}$$

11.1.2 *Total Inert Content, %:*

$$C = \sum_{i=1}^{n} x_i \qquad \textit{Equation 11.2}$$

where:

X = percent inert by type, % g g^{-1},
i = inert type, (e.g., hard plastics, metal, glass, stones, wood chips, etc.),
A = oven dry weight of recovered inert, g,
B = oven dry weight of the 250 cm^3 compost sample aliquot, g, and
C = sum of inerts, tally of percentages for all inert classes, % w/w.

03.06 METHODS SUMMARY

12. Report

12.1 *Report the following information:*

12.1.1 Express results for each inert type identified in the compost sample on an oven-dried basis to the nearest ±0.1 % g g^{-1}.

12.1.2 Express results for the sum of inerts in the compost on an oven-dried basis to the nearest ±0.1 % g g^{-1}.

13. Precision and Bias

13.1 *Percent Glass Shards, Metal Fragments and Hard Plastics:*

13.1.1 *Method 03.06-A Glass Shards, Metal Fragments and Hard Plastics Wet Sieving Technique—* The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

14. Keywords

14.1 glass; metal; plastic; inerts; sieve

Test Method: Process to Reduce Sharps.							**Units:** %, g g^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	03.07	03.07		03.07	03.07			

03.07 PROCESS TO REDUCE SHARPS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 Sharps are stainless steel hypodermic needles, and steel sewing needles and straight pins that can pose a human and animal puncture hazard with unprotected exposure. Although some of these items can harbor pathogens, possible infection will be eliminated by the Process to Further Reduce Pathogens (PFRP, US EPA 40 CFR Part 503). Long term, sharps can be oxidized in soil and introduce trace elements. Sharps are inert material and may be considered as a separate category of inerts, (e.g., sharps).

1.2 The Process to Reduce Sharps (PRS) is a recommended process standard drafted by the USCC Standards and Practices Committee, rather than a recommended product standard and is subject to peer review.

1.3 When appropriate, compost product intended for sale or distribution shall be treated for the effective removal of sharps, including steel sewing needles and straight pins, stainless steel hypodermic needles, wire snip ends, and metal shavings.

2. Summary of Test Methods

2.1 A suitable laboratory method to detect sharps has not been devised.

2.2 *Recommended Process*—Treatment shall take place after product particle size and texture is reduced to a fine, uniform soil-like material - characteristic of stable to very stable compost and may be by a combination of the following provisions:

2.2.1 by subjecting product to magnetic separation devices designed to remove ferrous items after compost curing; and

2.2.2 by subjecting product to an eddy current device designed to remove metallic materials after compost curing following ferrous separation; or

2.2.3 by sifting cured product through a physical separation device, such as an air flotation fluidized bed separator (destoner) equipped with a punched ±2.5 mm round, or equally effective, hole-size deck screen, designed for removal of stainless steel hypodermic needles.

3. Keywords

3.1 hypodermic needle; needles; Process to Reduce Sharps; PRS; sharps; straight pins

Test Method: Man Made Inerts. One Method.						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	03.08-A				03.08-A	03.08-A	03.08-A	03.08-A

03.08 MAN MADE INERTS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the quantification of man-made inert materials in compost.

1.1.1 *Method 03.08-A Classification of Inerts.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 02.01-B Sample Sieving for Aggregate Size Classification.

Method 02.01-C Man-Made Inert Removal.

Method 03.05 Film Plastics

Method 03.06 Glass Shards, Metal Fragments and Hard Plastics

3. Terminology

3.1 *glass, n*—Any of a large class of materials with highly variable mechanical and optical properties that solidify from the molten state without crystallization, are typically made by silicates fusing with boric oxide, aluminum oxide, or phosphorus pentoxide, are generally hard, brittle, and transparent or translucent, and are considered to be supercooled liquids rather than true solids.

3.2 *man-made inerts, n*—includes synthetic textiles, plastics, metal objects, and glass.

3.3 *metal, n*—Any of a category of electropositive elements that usually have a shiny surface, are generally good conductors of heat and electricity, and can be melted or fused, hammered into thin sheets, or drawn into wires. Typical metals form salts with nonmetals, basic oxides with oxygen, and alloys with one another. An alloy of two or more metallic elements. An object made of metal.

3.4 *plastics, n*—Any of various organic compounds produced by polymerization, capable of being molded, extruded, cast into various shapes and films, or drawn into filaments. Objects made of plastic.

3.5 *wood chips, n*—A compost bulking agent or mulching material as chips > 9.5 mm (⅜ in.). Wood chips with particle size < 9.5 mm are considered to be compost.

4. Summary of Test Methods

4.1 *Method 03.08-A Classification of Inerts by Sieve Size*—Inerts may be classified for each sieve size fraction. Size fractions can include 50, 25, 16, 9.5, 6.3, 4 and 2 mm. Inerts are hand sorted and classified for each size fraction.

4.1.1 Man-made inert content greater than 4 mm are determined by passing four replicates of 250 cm^3 oven dried (70±5C°) samples of the compost through a 4-mm sieve. Material remaining on the sieve is visually inspected and sorted. Clearly identifiable man-made inerts, including glass, metal, and hard plastic, are separated.

4.1.2 After the inerts > 4 mm and sharps > 2 mm are removed, the sample is milled and an aliquot is used for heavy metals analysis and other tests that require finely milled sample aliquots.

5. Significance and Use

5.1 Glass and metal shards 4-13 mm pose a human and animal hazard with unprotected exposure or through direct ingestion. Metal fragments may pose the same hazard, and are a potential source of trace metal contamination. Hard plastic is an aesthetic concern and can modify the physical properties of the soil when present in significant quantities.

5.2 Soil is composed of particles of material equal to or less than 2 mm in size. Particles larger than 2 mm are either gravel or foreign matter. Foreign matter of interest in compost as a soil amendment is man-made inert material greater than 4 mm. Foreign matter particle sizes larger than 4 mm is an aesthetic concern, possibly a safety concern, and may impact soil physical and chemical properties.

6. Interference and Limitations

6.1 Interference and limitations have not been determined for this test. Information is being sought to formulate a Interference and Limitations statement.

7. Sample Handling

7.1 Perform inert screening tests on size-classed samples (Method 02.02-B). If elemental analysis will be performed on samples following inert classification and removal, store material in a sealed container at room temperature to minimize sample moisture changes.

7.2 The < 9.5 mm fraction is air dried at a temperature of 36°C to minimize loss of volatile compounds and elements such as mercury.

Test Method: Man Made Inerts. Classification of Inerts.							**Units:** % g g^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	02.02-B				02.02-B	02.02-B	02.02-B	02.02-B
	02.02-C				02.02-C	02.02-C	02.02-C	02.02-C
							03.05-A	03.05-A
							03.06-A	03.06-A

03.08-A CLASSIFICATION OF INERTS

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. TMECC Methods:

8.1 Method 02.02-B Sample Sieving for Aggregate Size Classification, and

8.2 Method 02.02-C Man-Made Inert Removal; or

8.3 Method 03.05-A Film Plastic Surface Area Determinations Using Digital Processing; or

8.4 Method 03.06-A Glass Shards, Metal Fragments and Hard Plastics Wet Sieving Technique.

Test Method: Total Solids and Moisture. One Method							**Units:** % g g^{-1} wet basis	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
03.09-A	03.09-A	03.09-A	03.09-A	03.09-A	03.09-A	03.09-A	03.09-A	03.09-A

03.09 TOTAL SOLIDS AND MOISTURE

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the determination of total solids and moisture content of feedstocks and composts.

1.1.1 *Method 03.09-A Total Solids and Moisture at 70±5°C.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 03.02 Ash.

2.2 *Other References:*

ASTM D 2974-87, Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils. *In* Annual Book of ASTM Standards, Vol. 04.08. (Re-approved 1995).

Cohen, I.R. 1973. Laboratory Procedure for the Preparation of Solid Waste Related Materials for Analysis. *In* Methods of Solid Waste Testing, EPA-6700-73-01. US EPA, Cincinnati, OH.

Soil Testing and Plant Analysis. Third Edition. R.L. Westerman, ed. 812 pp. 1990. SSSA. No. 3 in SSSA Book Series.

SM 2540 B, Total Solids Dried at 103-105°C. 1992. Part 2000, Physical and Aggregate Properties. *In* Standard Methods for the Examination of Water and Wastewater. 18th Edition. 1992.

3. Terminology

3.1 *ash, n*—The inorganic matter, or mineral residue of total solids that remains when a compost or feedstock is combusted at 550°C in the presence of excess air; *Fixed Solids*, % g g^{-1}.

3.2 *biodegradable volatile solids, n*—The biodegradable portion of total solids that volatilizes to carbon dioxide and other gasses when a compost or feedstock is combusted at 550°C in the presence of excess air, % g g^{-1}.

3.3 *fixed solids, n*—The inorganic matter, or mineral residue of total solids that remains when a compost or feedstock is combusted at 550°C in the presence of excess air; *Ash*, % g g^{-1}.

3.4 *moisture content, n*—The liquid fraction (percentage) of a compost or feedstock that evaporates at 70±5°C, % g g^{-1}.

3.5 *total solids, n*—The solid fraction (percentage) of a compost or feedstock that does not evaporate at 70±5°C, which consists of fixed solids, biodegradable volatile solids, and volatile solids that are not readily biodegradable, % g g^{-1}.

4. Summary of Test Method

4.1 *Method 03.09-A Total Solids and Moisture at 70±5°C*—An aliquot of a well-mixed, as-received, bulk sample is weighed, oven dried at 70±5°C to steady state and re-weighed. The remaining dry solids fraction represents the total solids, and the evaporated fraction represents percent moisture.

4.1.1 Total solids and percent moisture of feedstock, in-process material, and finished compost products are determined using the same procedures.

4.1.2 The total solids is a measure of the amount of organic plus inorganic solid material present in a sample relative to the bulk sample weight. In contrast, percent moisture indicates the sample moisture relative to the bulk sample weight.

4.1.3 This test is performed in conjunction with all methods that are reported on a dry weight basis.

5. Significance and Use

5.1 Feedstocks for compost, in-process materials and compost are present in two phases: solid and liquid.

5.1.1 Total solids, or dry matter, in composting feedstocks and compost includes combustible or biodegradable organic material, or volatile solids and inorganic material, or fixed solids making up the ash remaining when organic matter is oxidized by combustion.

5.1.2 Total solids does not include trash that is removed during feedstock recovery operations or during compost finishing. Trash includes stones, carbonate concretions, and manufactured inert materials over 4 mm, such as metal fragments, glass shards, sharps, leather, textiles, hard plastic and film plastic.

5.2 Inorganic content of compost should be measured on a dry weight basis when the product is ready for marketing. Reporting certain chemicals in biosolids compost at the time of marketing is required by EPA Chapter 40, CFR Part 503. To insure valid comparisons for chemical concentrations in two or more products, the composts being compared must be at the same level of biological stability.

6. Interference and Limitations

6.1 Compost samples are oven dried at 70±5°C for approximately 18 h to 24 h, until weight change diminishes to nil. At temperatures above 70±5°C, there is increased weight loss due to volatile loss of compounds such as CO_2 in addition to water.

6.2 Negative errors in volatile solids can be produced by loss of volatile matter during drying. Errors associated with the volatile solids determinations are increased when low concentrations of volatile solids are observed with high fixed solids. In such cases, measure for suspect volatile components by another test, for example, total organic carbon.

6.3 Composts that do not contain significant levels of semi-volatile compounds will yield identical total solids results when dried at either 70±5°C or 103°C - 105°C. The latter temperature is recommended for soils and biosolids; it significantly reduces drying time. This rapid method with higher drying temperature is not recommended for use with all composts; compost often contains significant levels of compounds that volatilize or evaporate above 75°C. Significant losses of these volatile compounds will distort reported concentrations of nutrients, metals and other parameters that are corrected to a dry weight basis.

7. Sample Handling

7.1 *Method A. Total Solids and Moisture at 70±5°C*—Perform this test on feedstocks, in-process and finished composts. The material may contain unclassified inert material.

7.1.1 Determinations are made at 70±5°C on a representative aliquot of unsieved or sieved bulk material and all sample size fractions of interest, including all sieve classes, and feedstocks.

7.1.2 This test is best performed in conjunction with sample sieving as outlined in Test Method 02.02-B.

Test Method: Total Solids and Moisture at 70±5°C							**Units:** % g g^{-1} wet basis	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
03.09-A	03.09-A	03.09-A	03.09-A	03.09-A	03.09-A	03.09-A	03.09-A	03.09-A

03.09-A TOTAL SOLIDS AND MOISTURE AT 70±5°C

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Apparatus for Method A

8.1 *Balance*—capable of weighing 100 g, with accuracy of ±0.001 g.

8.2 *Desiccator Cabinet*—vacuum with desiccant tray containing color indicator of moisture concentration or an instrument indicator.

8.3 *Evaporation Dish*—minimum capacity of 150-mL, heat-resistant borosilicate glass is recommended and will serve as an appropriate vessel for both total solids and moisture, and LOI OM determinations.

8.4 *Forced-Air Drying Oven*—vented, set at 70±5°C (do not microwave).

9. Reagents and Materials for Method A

9.1 *None required.*

10. Procedure for Method A

10.1 *Measure As-Received Sample Weight:*

10.1.1 Transfer 50 cm^3 aliquot of prepared finished compost material to a tared 150-mL open beaker.

10.1.2 Weigh and record gross weight of beaker and sample, subtract beaker tare weight from gross weight to determine net weight of moist sample, ±0.001 g.

NOTE 1A—For feed stock samples and in-process samples increase the volume of material to approximately one gal and transfer this to a tared sample container.

10.2 *Determine Oven-Dried Sample Weight:*

10.2.1 Place open beaker containing the as-received moist sample aliquot into the forced-air drying oven preheated to 70±5°C. Dry the sample for 18 h to 24 h until weight change due to moisture loss diminishes to nil.

10.2.2 Place the oven-dried sample in desiccator and cool to ambient laboratory temperature, approximately 23°C.

10.2.3 Weigh and record the gross weight of beaker and dry sample.

10.2.4 Subtract the mass of the beaker from the gross weight to determine sample net oven-dried weight.

10.3 *Total Solids and Moisture:*

10.3.1 Calculate total solids , % g g^{-1}, wet basis,

10.3.2 Calculate sample moisture, % g g^{-1}, wet basis.

11. Calculation for Method A

11.1 *Calculate Total Solids and Percent Moisture:*

$$TS = dw \div A \times 100 \qquad \textit{Equation 11.1.1}$$

$$M = 1 - [dw \div A] \times 100 \qquad \textit{Equation 11.1.2}$$

where:

TS = percentage solid material in sample, wet basis, % g g^{-1},

M = percentage moisture in sample, wet basis, % g g^{-1},

dw = net dry weight, oven at 70±5°C, g, and

A = net sample weight at as-received moisture, g.

03.09 METHODS SUMMARY

12. Report

12.1 *Total Solids*—reported as a percentage of dry solids contained in an as-received sample, ±0.1 % g g^{-1}, wet basis.

12.1.1 The ratio for total solids (oven-dried weight ÷ as-received weight) is used to correct reported values (concentration, mass, volume, etc.) to standard moisture content on an oven-dry weight basis. No correction need be made for variations in barometric pressure (altitude).

12.2 *Moisture Content*—reported as a percentage of as-received weight, ±0.1 % g g^{-1}, wet basis.

13. Precision and Bias

13.1 High relative precision can be attained by thoroughly blending and mixing the entire sample in a closed sample container prior to aliquoting the test sample. Accuracy of the test is a function of the sampling strategy employed in the field. If an adequate number of subsamples is collected and properly mixed at the time of collection, the composite sample sent to a laboratory will represent the compost in question. Refer to section 02.01 Field Sample Collection.

13.2 *Total Solids and Moisture:*

13.2.1 *Method 03.09-A Total Solids and Moisture at 70±5°C*—The precision of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

13.2.1.1 Precision was determined using ten subsamples taken from a field composite sample for each of three sites for two sampling periods, (1993).

Table 03.09-A1 Total Solids, % as-received wet weight basis. Precision estimates for < 6.3 mm as-received municipal solid waste compost material, (1993).

Median	*Std Dev*	*% CV*	*Number of Samples*
58.71	1.16	2.0	10
61.29	0.65	1.1	10
70.38	0.00	0.0	10
61.19	0.43	0.7	10
66.78	0.95	1.4	10
76.07	0.25	0.3	10

Note 2A—Coefficient of Variation, %CV = Standard Deviation ÷ Mean × 100.

Table 03.09-A2 Moisture Content, % as-received wet weight basis. Precision estimates for < 6.3 mm as-received MSW compost material, (1993).

Median	*Std Dev*	*% CV*	*Number of Samples*
37.44	0.10	0.3	10
38.71	0.65	1.7	10
26.26	0.59	2.2	10
38.81	0.43	1.1	10
33.22	0.95	2.8	10
23.93	0.25	1.0	10

14. Keywords

14.1 total solids; moisture; oven-dry; oven-dried; as-received; ash; fixed solids; evaporate; volatile solids; biodegradable volatile solids

Test Method: Water Holding Capacity. Five Methods.						**Units:** % w w^{-1} dw basis		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
	03.01-A	03.01-A	03.01-A	03.01-A				
		03.01-B		03.01-B		03.01-B		
	03.01-C	03.01-C	03.01-C	03.01-C		03.01-C		
								03.01-D
				03.01-E	03.01-E	03.01-E		

03.10 WATER HOLDING CAPACITY

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This test covers the measurement of water-holding capacity of compost.

1.1.1 *Method 03.10-A, refer to Method 03.01-A Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Unsieved).*

1.1.2 *Method 03.10-B, refer to Method 03.01-B Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Sieved)*

1.1.3 *Method 03.10-C refer to Method 03.01-C Field Density, Free Air Space and Water-Holding Capacity*

1.1.4 *Method 03.10-D Bulk Density and Water-Holding Capacity of Compost Material, Modified ASTM D 2980-71*

1.1.5 *Method 03.10-E Quick-Test to Approximate Water-Holding Capacity of Compost*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Reference Documents

ASTM D 2980-71. 1971. American Society of Testing Materials, Philadelphia, PA. 19103.

Haug, Roger T., The Practical Handbook of Compost Engineering, Lewis Publishers, 1993.

Methods of Solid Waste Testing. Laboratory Procedure for the Preparation of Solid Waste and Related Materials for Analysis. US EPA. 1973. Office of Research and Monitoring. EPA-6700-73-01, Part I. p 3.

Water holding Capacity, Volume Mass and Air Capacity of Water-Saturated Peat (ASTM D 2989-71). p 77. *In* Peat Testing Manual. National Research Council of Canada, Technical Memorandum No. 125. 1979.

3. Summary of Test Methods

3.1 *Method 03.01-A Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Unsieved)*—A compost sample taken from the pile, as is, of known volume and mass is systematically transferred to a graduated beaker and bulk density is determined. The compost is saturated with water and excess water is drained. Changes in compost volume and mass, and the ratio of water retained relative to the amount of drained water provide a means for estimating compost bulk density, porosity/pore water volumes and free airspace, and water holding capacity.

3.2 *Method 03.01-B Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Sieved)*—A compost sample taken from the pile, as is, of known volume, is sieved and systematically transferred to a graduated beaker and bulk density is determined. The compost is saturated with water and excess water is drained. Changes in compost volume and mass, and the ratio of water retained relative to the amount of drained water provide a means for estimating compost bulk density, porosity/pore water volumes and free airspace, and water holding capacity.

3.3 *Method 03.10-C Field Density, Free Airspace and Water-Holding Capacity*—Compost samples are

systematically transferred to a bucket and weighed to approximate compost density. The same bucket containing compost is filled with water and weighed, where the volume equivalent of the added water is used to approximate compost pore space. The water then drained and the remaining water-saturated compost is weighed to approximate the water-holding capacity of the compost.

3.4 *Method 03.01-D Bulk Density, Water-Holding Capacity, and Air Capacity of Compost Material, Modified ASTM D 2980-71*—A sample of known volume and mass of material is transferred to a burette and saturated with water. Excess water is drained. The resulting sample weight gain due to retained moisture represents the relative water holding capacity of the material.

3.4.1 Saturated volume weights, water holding capacity, bulk density, compost volumes and air volume are determined on both weight and volume basis from these data.

3.4.2 The bulk density test result is reported as g cm^{-3} with the weight expressed on a 70±5°C dry weight basis, where volume is measured after the sample is packed as indicated; the dry weight adjustment is based on a separate aliquot of compost material.

3.5 *Method 03.10-E Quick-Test to Approximate Water-Holding Capacity of Compost*—A *Quick-Test* to approximate water-holding capacity is performed on sieved or unsieved, as-received finished compost or finely milled feedstocks. A sample is systematically saturated with water.

3.5.1 This method provides a quick estimate for compost water holding capacity, and requires minimal apparatus and sample preparation. Approximately 1 h is required to complete a run, whereas 5 to 24 h are required to perform the methods described under TMECC 03.10-A through 03.10-D.

4. Significance and Use

4.1 *Methods 03.10-A and -B, refer to Methods 03.01-A and -B Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Unsieved)*—Quick-Test to determine volume weights (bulk density), porosity/pore space, water holding capacity, and air capacity (free air space) performed on unsieved, as-received compost material.

4.1.1 This method provides a quick estimate for compost bulk density, porosity/pore space percent, free airspace percent, and water-holding capacity. Approximately 5 h are required to complete a run, whereas 24 h are required to perform the method described under TMECC 03.10-C and 03.10-D.

4.2 *Method 03.10-C Field Density, Free Airspace and Water-Holding Capacity*—This test is designed to provide a rough approximation for field diagnostic use only.

4.3 *Method 03.10-D Bulk Density and Water-Holding Capacity of Compost Material, Modified ASTM D 2980-71*—Analytical test to determine volume weights (bulk density) and water-holding capacity, performed on sieved, as-received compost material.

4.4 *Method 03.10-E Quick-Test to Approximate Water-Holding Capacity of Compost*—this test provides a very quick estimate for water-holding capacity. Approximately 1 h is required to complete a run.

5. Interference and Limitations

5.1 In-process compost sample moisture should range from 45-60% (wet basis). Finished product compost sample moisture should range from 40-50% (wet basis). Excessively moist samples will compact during preparation, and inflate bulk density estimates and deflate water-holding capacity estimates. Excessively dry samples are often difficult to saturate with water (sometimes hydrophobic).

CAUTION !—Excessively moist or dry initial in-process compost samples will provide invalid results.

5.2 *Method 03.01-A, -B and -C*—These methods do not use vacuum to assist water extraction from water filled pores (*c.f.* Method D). Therefore, incomplete removal of free water *(water-filled air space)* from air pore space will deflate air capacity estimates and inflate water-holding capacity estimates.

5.3 *Method 03.01-D Bulk Density, Water-Holding Capacity, and Air Capacity of Compost Material, Modified ASTM D 2980-71*—This is a modified version of ASTM D 2980-71 for Volume Weights, Water-Holding Capacity, and Air Capacity of Water-Saturated Peat Material and considers specific characteristics of compost rather than peat. Because the physical character of compost is similar to that of peat, the method has been accepted for physical determinations of compost. ASTM D 2980-71 calls for the test to be performed on a sample that has passed through a 4-mm screen. A sieve size no smaller than 9.5 mm is recommended for compost. Modifications to the recommended apparatus design from the published method were made to enhance the durability of the screen mount on the burette base.

5.3.1 The Volume Weight procedure given in this ASTM D 2980-71 includes a density measurement and is sometimes recommended as a determination method for the bulk density of compost. ASTM Method, D 4531-86 for Bulk Density of Peat and Peat Products,

was developed for bulk density determinations on undisturbed core samples of peat and is not recommended for loose compost samples.

5.3.2 ASTM D 2980-71 performs well with compost materials with similar physical characteristics to those of peat. If the compost material being tested is similar to soil, i.e. high bulk density, > 0.7 g cm^{-3}, rather than peat, a negative air space value will result.

5.3.3 Film plastics can block the screen at the base of the upper burette. If this occurs, water may not drain, or will drain too slowly. To correct this situation, simply grasp the sample burette above and below the screen and slowly twist the burette, just breaking the seal formed by the plastic. Return the burette to the stand and proceed. Repeat this process until water flows.

6. Sample Handling

6.1 *Method 03.10-A Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Unsieved)*—Material used in this test should represent as-received, unseived, in-process compost product at 45-60% moisture (wet weight basis).

6.2 *Method 03.10-B Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Sieved)*—Material used in this test should represent in-process compost product at 45-60% moisture (wet weight basis). As-received sample aliquots should pass a 9.5-mm sieve.

6.3 *Method 03.10-C Field Density, Free Airspace and Water-Holding Capacity*—Material used in this test should represent unseived, in-process compost product at 45-60% moisture (wet weight basis).

6.4 *Method 03.01-D Bulk Density, Water-Holding Capacity, and Air Capacity of Compost Material, Modified ASTM D 2980-71*—Material used in this test should represent the marketable compost product. The sample aliquot source should be the working sample stored at ~4°C. Avoid performing this test on material that is too wet or too dry. Follow sample preparation protocol outlined in that section. Material used in this test should represent finished compost product at 45-60% moisture (wet weight basis). Moisture content varies with water-holding capacity and bulk density, and water-holding capacity decreases as compost density increases.

6.5 *Method 03.10-E Quick-Test to Approximate Water-Holding Capacity of Compost*—Material used in this test should represent unseived, in-process compost product at 45-60% moisture (wet weight basis).

Test Method: Water Holding Capacity. Refer to Methods 03.01-A and 03.01-B Quick-Test for Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost						**Units:** % w w^{-1} dw basis		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
	03.01-A	*03.01-A*	*03.01-A*	*03.01-A*				
		03.01-B		*03.01-B*		*03.01-B*		

03.10-A AND 03.10-B QUICK-TEST FOR BULK DENSITY, POROSITY/PORE SPACE, FREE AIRSPACE AND WATER HOLDING CAPACITY OF UNSIEVED (OR SIEVED) COMPOST

REFER TO 03.01-A AND 03.01-B QUICK-TEST FOR BULK DENSITY, POROSITY/PORE SPACE, FREE AIRSPACE AND WATER HOLDING CAPACITY OF COMPOST.

Test Method: Water Holding Capacity. Refer to Method 03.01-C Field Density, Free Airspace and Water-Holding Capacity						**Units:** % w w^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
	03.01-C	*03.01-C*	*03.01-C*	*03.01-C*				

03.10-C FIELD DENSITY, FREE AIR SPACE AND WATER-HOLDING CAPACITY

REFER TO 03.01-C FIELD DENSITY, FREE AIR SPACE AND WATER HOLDING CAPACITY.

Test Method: Water Holding Capacity. Bulk Density, Water-Holding Capacity, and Air Capacity of Compost, Modified ASTM D 2980-71							**Units:** %, w w^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
								03.01-D

03.10-D BULK DENSITY AND WATER-HOLDING CAPACITY, OF WATER-SATURATED COMPOST, MODIFIED ASTM D 2980-71

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

7. Apparatus for Method D

7.1 *Funnel*—15 cm (6 in.) diameter mouth, 2.5 cm (1 in.) diameter delivery stem.

7.2 *Rubber Ball*—3.2 cm (1⅜ in.) diameter with a hardness of 50-55 shore by "A" durometer measurement, drilled hole 1.2 cm (⅜ in.) diameter, 1.8 cm (¾ in.) deep.

7.3 *Rubber Tubing*—7.5 cm (3 in.) length, 2.5 cm (1 in.) diameter.

7.4 *Screen Material and Teflon Collar*—stainless steel 16-mesh, cut round to fit inside the upper machined lip of the Teflon collar. Teflon Collar (see diagram 12.4 in Fig 03.10-D1).

7.5 *Rubber Stoppers*—3 No. 6½, fitted with glass tubing.

7.6 *Tubing*—one 5 cm (2 in.) length, one 45 cm (18 in.), one 90 cm (36 in.) length, 1.1 cm (⅜ in.) i.d., Tygon, rubber or other flexible tubing.

7.7 *Tubing*—glass 0.6 cm (¼ in.) o.d., rubber/plastic 0.6 cm (¼ in.) i.d. One length of glass tubing to be bent U shaped with a short and long extension.

7.8 *Pinch-clamps*—two, screw adjusting.

7.9 *Burettes*—two 250-mL with 1-mL subdivisions, or one burette and an acrylic cylinder of similar length and diameter to the burette.

7.9.1 *Burette Modification*—cut burette at the 250 mL mark (see Fig 03.10-D1.12.9.1).

7.9.2 *Optional Acrylic Cylinder*—similar length and diameter to the 250-mL burette (see Fig 03.10-D1.1.9.2).

7.10 *Transfer Cup*—one 20-mL cup, plastic or glass.

7.11 *Evaporation Dish*—one 150-mL beaker, Pyrex or other heat resistant material.

7.12 *Analytical Balance*—capable of weighing 0.01 to 1 kg, accurate to ±0.01 g.

7.13 *Bottle or Pan*—20-L (5-gal), plastic or other material.

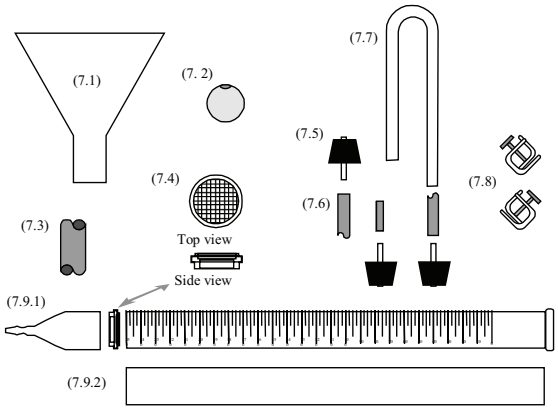

Fig 03.10-D1 Apparatus for analytical determinations of bulk density, water-holding capacity, and air capacity of compost material, Modified ASTM D 2980-71

COMMENT—Individual apparatus components illustrated in Fig 03.10-D1 are numerically labeled and correspond to the apparatus listing from section 7.

8. Reagents and Materials for Method D

8.1 *Water Source*—tap water, low volume to maintain constant flow rate required for 12 h - 18 h intervals.

9. Procedure for Method D

9.1 *Burette Assembly*—Fit machined Teflon collar with screen and place between the cut ends of the burette. Firmly couple the two pieces of burette together by slipping the 7.5 cm (3 in.) length of 2.5 cm (1 in.) diameter rubber tubing over joint to ensure a water/air tight seal.

9.2 *Tare Burette*—Weigh empty burette fitted with the 16-mesh screen and Teflon collar.

9.3 *Transfer Material to Burette*—Refer to FIG D2.1 for illustration.

9.3.1 Work rapidly to minimize evaporative water loss, add the sample to the burette through funnel.

9.3.2 Fill the burette to 20 to 25 cm (7.5 to 10 in.) with compost by adding approximately 20 cm^3 portions.

9.3.3 Upon the addition of each 20 cm^3 portion, allow the burette fitted with rubber ball to drop vertically onto a hard surface from a height of 15 cm (6 in.).

9.3.4 Allow the burette assembly to bounce only once after the 15-cm vertical drop. This practice will minimize non-uniform compaction of compost through burette.

9.3.5 Record volume of filled burette, V_r, ±0.5 cm^3.

9.4 *Record As-Received Weight*—After filling and packing burette, remove the rubber ball from the burette tip and record the weight of the filled burette, W_r, ±0.1 g.

9.5 *Moisture Content and Total Solids*—Determine sample moisture on a parallel aliquot of compost taken from the working sample.

9.5.1 Transfer approximately 50 cm^3 of sample to a tared 150-mL beaker.

9.5.2 Weigh and record as-received weight, ±0.01 g.

9.5.3 Allow sample to oven dry in a forced-air oven at 70±5°C for approximately 1.5-2 d until moisture loss diminishes to nil, and record the oven dry weight, W_d, ±0.01 g.

9.6 *Burette Stand (Rack) Preparation*—Refer to FIG 03.10-C2.2 for illustration.

9.6.1 Position the two burettes vertically onto a holding device near a drain, the burette containing the sample above, the empty burette below.

9.6.2 Place a 20-L (5-gal) container filled with deionized water above the burette assemblies.

9.6.3 Construct a siphon device by bending a piece of 6 mm (¼ in.) glass tubing into a U-shape with the longer length extending to the bottom of the 20-L (5-gal) container and a short length bent to fit over the container outside lip. Refer to Fig 03.10-D1 for apparatus illustrations.

9.6.4 Fit a 30 to 60 cm (12 to 24 in.) length of 6 mm (¼ in.) i.d. soft plastic or rubber tubing onto the short end of the glass tubing. To the lower end of the flexible tubing, fit a No. 6½ rubber stopper using a short piece of glass tubing [7.5 cm (3 in.); 6 mm (¼ in.) o.d.].

9.6.5 The stopper is then fitted tightly into the top of the upper burette.

9.6.6 Clamp the tubing of this siphon device.

9.6.7 Fit a 7.5-10 cm (3-4 in.) piece of 6 mm (¼ in.) i.d. flexible rubber or plastic tubing with a pinch clamp to the delivery end of the upper burette. As above, fit a No. 6½ rubber stopper using a 7.5 cm length of glass tubing with 6 mm o.d. (3 in. length; ¼ in. o.d.) to the lower end of the flexible tubing. The stopper is then fitted tightly into the top of the lower burette.

9.7 *Saturate Sample with Water:*

9.7.1 Attach a short piece of rubber tubing [approximately 10 cm (4 in.) length] fitted with a screw-type of pinch clamp to the bottom delivery end of the burette.

9.7.2 With both pinch clamps released, pass water from the 20-L (5-gal) pan through the sample for 1 h or until the sample appears well wetted.

9.8 *Stabilize Water Flow for 24 h*—Refer to illustration 2 in Fig 03.01-D2.

9.8.1 After the initial soaking, regulate the water flow through the column to 1 mL per second by adjusting the upper pinch clamp at the delivery end of the burette.

NOTE 1C—The in-flow of water should be about equal to the out-flow, and one drop should equal 1 mL.

9.8.2 Allow water to flow through the sample for not less than 24 h, maintaining a water reservoir over the sample at all times.

9.9 *Remove Excess Water*—After saturating the sample, close both clamps and let the sample settle in water for about 5 min to allow excess water to drain (accumulated water above the sample surface).

9.10 *Drain Sample*—Refer to illustration 3 in Fig 03.01-D2.

9.10.1 Clamp the tubing at the delivery end of the lower cylinder.

9.10.2 Fill the lower cylinder with water.

9.10.3 Seal both the burette and lower cylinder (lubricating with glycerol) to prevent air leaks.

NOTE 2D—An acrylic cylinder of similar dimensions is used in place of the lower burette used in the ASTM publication.)

9.10.4 Drain the water by removing the clamped stopper from the mouth of the upper burette and the clamps from both ends of the apparatus. (Refer to illustration 3 in Fig 03.01-D2).

NOTE 3D—The water draining from the lower burette (or cylinder) creates a vacuum that pulls moisture from the compost material in the upper burette. The suction is equivalent to about 38 cm (15 in.) of water. Ensure that all stoppers are well seated to prevent air leaks and maintain a standard vacuum across samples.

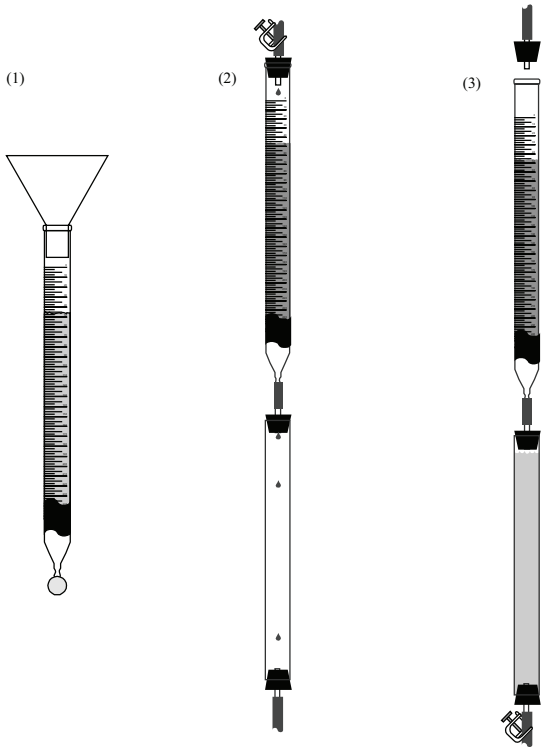

Fig 03.10-D2 Procedures: (1) filling and packing burette, (2) saturating sample, and (3) final draining of burette.

9.11 *Measure and Record Weight and Volume*—Measure the height of the wet compost. The height should be 19-25 cm (7.5 to 10 in.). Determine and record the volume (±0.1 mL) and weight of compost filled burette, ±0.1 g.

9.12 *Repeat Steps 14.7 through 14.11 until Result is Consistent:*

9.12.1 Wet the sample again by passing water through it for not less than 1 h.

9.12.2 Drain again by suction, record the volume, and re-weigh.

NOTE 4D—The original method calls for this sequence of steps to be repeated until consistent results are obtained. When this test was applied to MSW compost, it was found that one re-wetting as given above is sufficient.

10. Calculations for Method D

10.1 *Bulk Density*—Calculate as-received and dry weight per as-received volume, and the saturated volume weight per as-received volume.

10.1.1 *As-Received Volume Weight:*

$$D_r = W_r \div V_r \qquad \textit{Equation 10.1.1}$$

10.1.2 *Oven-Dried Volume Weight:*

$$D_d = W_d \div V_r \qquad \textit{Equation 10.1.2}$$

10.1.3 *Saturated Volume Weight:*

$$D_w = W_w \div V_r \qquad \textit{Equation 10.1.3}$$

where:

D_r = density of sample on as-received basis, kg m^{-3},
D_d = density of sample on oven-dried basis, kg m^{-3}, (*reporting unit of measure*),
D_w = density of water saturated sample after draining, kg m^{-3},
V_r = as-received volume of sample, cm^3,
W_d = weight of dried sample = $W_r \times [(100.0 - M) \div 100.0]$, g,
W_r = weight of test sample as-received, g,
W_w = weight of wet sample after final draining, g, and
M = wet weight based moisture content, dried at 70±5°C, % g g^{-1}.

NOTE 5D—ASTM calls for 105°C ODW with peat. This higher temperature (105°C) is not recommended for compost.

10.2 *Water-Holding Capacity:*

10.2.1 Weight basis:

$$W = (W_w - W_d) \div W_d \times 100 \qquad \textit{Equation 10.2.1}$$

10.2.2 Volume basis:

$$V = (W_w - W_d) \div V_r \times 100 \qquad \textit{Equation 10.2.2}$$

where:

W = water holding capacity as a percentage of weight, v w^{-1},
V = water holding capacity on a volume basis, assuming 1 g of moisture = 1 cm^3 of moisture = 1 mL of moisture, % v v^{-1},
W_d = weight of dried sample = $W_r \times [(100 - M) \div 100]$, g,
W_r = as-received weight of test aliquot g,
W_w = wet weight of saturated test aliquot, g,
V_r = as-received volume of sample, cm^3, and
M = moisture content, dried at 70±5°C, % g g^{-1} (wet weight basis).

Test Method: Water Holding Capacity. *Quick-Test* to Approximate Water-Holding Capacity of Compost							**Units:** % w w^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
				03.01-E	03.01-E	03.01-E		

03.10-E *QUICK-TEST* TO APPROXIMATE WATER-HOLDING CAPACITY OF COMPOST

SUBMITTED BY—Thomas R. Halbach; Department of Soil, Water, and Climate; University of Minnesota, St. Paul.

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

11. Apparatus for Method E

11.1 *burette*—100-mL, graduated.

11.2 *burette stand*—fitted with two burette clamps.

11.3 *funnel*—10-cm diameter mouth, stem length of approximately 10 cm, glass.

11.4 *ring stand*—used to support funnel, fitted with clamp for attaching to stand.

11.5 *graduated cylinder*—100-mL, 0.1 mL increments.

12. Reagents and Materials for Method E

12.1 *filter paper*—10-cm diameter disc, Wattman # 4 or equal.

12.2 *water*—deionized, 17 MΩ minimum standard.

13. Procedure for Method E

13.1 *Apparatus Assembly*—Attach burette to stand; adjust burette height to provide sufficient space for both funnel and graduated cylinder; position ring support on stand so funnel is directly below the delivery end of the burette; fasten the funnel to the burette stand; place the graduated cylinder below the stem of the funnel.

13.2 *Preparation*—Fill burette with 100 mL of water; record weight of dry filter paper (P_d); moisten filter paper and record weight of wet paper (P_w); fold and place wet filter paper in funnel; analytically transfer 50 cm^3 of compost at as-received moisture into the funnel, onto the wet filter paper; tamp compost sample very gently to ensure lateral displacement of moisture.

13.3 *Initiate Run*—Carefully open burette stop-cock to allow water to fall directly onto the center of the compost sample at a rate of one drop sec^{-1}; use the graduated cylinder to capture water that passes through funnel; continue for approximately 100 min.

13.4 *Determine Compost Moisture Content:*

13.4.1 After the sample is subjected to water-drops for 100 min, remove the sample and the filter paper from the funnel; measure and record the mass of the wet sample including paper filter (M_w); oven-dry the wet sample at 105°C for approximately 1.5 h and record the dry weight (M_d); calculate the sample moisture content; compensate for weight gain attributable to filter saturated paper.

NOTE 1E—The author calls for oven drying the sample at 105°C. This decreases a required drying time from about 2 d for the standard drying temperature of 70±5°C to only 1.5 h.

14. Calculations for Method E

14.1 Calculate mass of water contained in sample at end of run:

$$W = [M_w - M_d] - [P_w - P_d] \qquad \textit{Equation 14.1}$$

14.2 *Calculate water-holding capacity (volume basis):*

$$WHC = W \div 50 \qquad \textit{Equation 14.2}$$

14.3 *Calculate water-holding capacity (weight basis):*

$$WHC = W \div [M_d - P_d] \qquad \textit{Equation 14.3}$$

where:

WHC = water-holding capacity, mL water cm^{-3} oven-dried compost (volume basis), or g water g^{-1} oven-dried compost (weight basis),

W = total amount of water held by 50 cm^3 compost sample, mL ≡ g,

M = mass of compost sample subjected to test (w=wet; d=dry), g,

$[P_w - P_d]$ = mass of water held in filter paper (w=wet; d=dry), g,

$[M_w - M_d]$ = total amount of water held in sample and filter paper, mL ≡ g,

$[M_d - P_d]$ = dry weight (determined at 105ºC) of the 50 cm^3 compost sample, and

50 = 50 cm^3 sample subjected to water drops.

03.10 METHODS SUMMARY

15. Report

15.1 *Water Holding Capacity*—Report oven drying temperature, sample moisture content and water-holding capacity on both a weight and volume basis.

15.1.1 *Weight Basis*—(%, from Method 03.10, Equation 10.2.1) a percentage oven-dry weight basis, ±0.1 % w w^{-1}, water:compost.

15.1.2 *Volume Basis*—(%, from Method 03.10, Equation 10.2.2) a percentage as-received volume weight basis, ±0.1 % v v^{-1}, water:compost.

15.2 *Method 03.10-D Quick-Test to Approximate Water-Holding Capacity of Compost*—Report water-holding capacity (water/compost): volume basis, ±0.5 % mL cm^{-3}; and weight basis, ± 0.5 mL g^{-3}, dw.

16. Precision and Bias

16.1 *Water Holding Capacity:*

16.1.1 *Method 03.10-A Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Unsieved)*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

16.1.2 *Method 03.10-B Bulk Density, Porosity/Pore Space, Free Airspace and Water Holding Capacity of Compost (Sieved)*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

16.1.3 *Method 03.10-C refer to Method 03.01-C Field Density, Free Air Space and Water-Holding Capacity*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

16.1.4 *Method 03.10-D Bulk Density, Water-Holding Capacity, and Air Capacity of Water-Saturated Compost, Modified ASTM D 2980-71*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

16.1.5 *Method 03.10-E Quick-Test to Approximate Water-Holding Capacity of Compost*—The precision and bias of this proposed test have not been determined. Data are being sought for use in developing a precision and bias statement.

17. Keywords

17.1 water-holding capacity

Test Method: Organic Carbon. One Method							**Units:** % g g^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.01-A	04.01-A	04.01-A	04.01-A				04.01-A

04.01 ORGANIC CARBON

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the measurement of total organic carbon content of composting feedstocks and compost.

1.1.1 *Method 04.01-A Combustion with CO_2 Detection.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 02.02 Laboratory Sample Preparation.

Method 03.09 Total Solids and Moisture.

2.2 *Reference Manuals and Research Institutions:*

Leco Corporation, 3000 Lakeview Dr., St. Joseph, MI 49085.

Canadian Centre for Mineral and Energy Technology (CANMET), 555 Booth St. Ottawa, Canada, KlAQGl.

Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, The Netherlands.

US EPA SW-846. Test Methods for Evaluating Solid Wastes. Chapter 5. Miscellaneous Test Methods. Method 9060A: Total Organic Carbon

2.3 *Literature Citations:*

Mathur, S.P., G. Owen, H. Dinel and M. Schnitzer. 1993. Determination of compost maturity. I. Literature review. Biol. Agric. Hort. 10:65-85.

Nelson, D.W., and L.E. Sommers. 1982. Total carbon, organic carbon, and organic matter. *In* A.L. Page *et al*. Methods of Soil Analysis. Part 2. 2nd ed. p 539-579.

3. Terminology

3.1 *organic carbon, n*—biologically degradable carbon containing compounds found in the organic fraction of compost. Sugars, starches, proteins, fats, hemicellulose, cellulose and lignocellulose are present in compost feedstocks which are biologically degraded during composting and curing. Other organic carbon forms that are generally not degraded biologically include petroleum and petroleum byproducts, such as plastics and contaminated oils. They can be degraded by physical means, (e.g., if the temperature is sufficiently high). The organic carbon fraction does not include inorganic carbonate concretions such as calcium and magnesium carbonates, (*c.f.*, Method 04.08 Inorganic Carbon).

3.2 *organic matter, n (OM)*—the sum of substances in a compost or soil that contain organic carbon; the total organic components in soil or compost including undecayed plant and animal tissues, their partial decomposition products, and the soil or compost biomass exclusive of living macrofauna and macroflora. Refer to Method 05.07-A Loss on Ignition Organic Matter.

3.3 *organic matter fractions, n (fulvic acid, humic acid, humin)*—complex mixtures of polymeric organic molecules that cannot be separated into homogeneous molecules and cannot be precisely defined in chemical terms. Fraction ratios vary directly with the strength of base and acid employed in the extraction/separation procedure. Refer to Method 05.07-B Humic Substances: Fulvic acid and humic acid extraction and characterization.

4. Summary of Test Methods

4.1 *Method 04.01-A Combustion with CO_2 Detection*—This method uses a carbon analyzer, (e.g., Leco CR-12), to determine total organic carbon in compost. The analyzer operates on the principle of total combustion of a sample in an oxygen-rich atmosphere of a 1370°C (2500°F) resistance furnace. The CO_2 produced by the combustion is swept into an oxygen stream through anhydrone tubes to scrub H_2O

vapor from the stream. The CO_2 stream is then fed into the infrared detector and the amount of CO_2 produced is measured.

5. Significance and Use

5.1 The total organic carbon content of compost originates from sugars, starches, proteins, fats, hemicellulose, cellulose and lignocellulose that are found in composting feedstocks and are degraded during composting and curing. During the microbial degradation of carbon compounds, carbon is transformed in the solid phase to humin, from the solid to liquid phase as humic carbon, and to the gaseous phase as carbon dioxide. Bacteria, actinomycetes and fungi are responsible for the degradation. Substances containing humic carbon include humic acid (HA) and fulvic acid (FA).

5.2 Total organic carbon represents the sum of all forms of organic carbon, both degraded and undegraded. Other carbon forms that are inorganic, (not organic), are carbonate concretions such as calcium and magnesium carbonates, (Method 04.08 Inorganic Carbon).

5.3 Compost stability generally increases as total organic carbon decreases. A diminishing presence of unstable carbon compounds is reflected in a lower total organic carbon measure and lower rates of microbial activity, (*c.f.*, Method 05.08-F Biologically Available Carbon).

6. Interference and Limitations

6.1 Unless accounted for, the presence of carbonates or non-biodegradable petroleum-based materials will inflate organic carbon determinations in a compost sample.

6.1.1 A sample that effervesces in dilute HCl contains significant amounts of carbonates that will inflate the carbon measurement.

6.1.2 A sample containing carbonates can be pretreated (leached) with acid to remove the carbonate carbon. Nitric acid is used to leach and volatilize carbonates.

6.1.3 Inert removal prior to total organic carbon determination will minimize inflated carbon values due to the combustion of petroleum-based materials such as film and hard plastics, (Method 03.05 Film Plastics; and Method 03.06 Glass Shards, Metal Fragments and Hard Plastics).

6.2 *Method 04.01-A Combustion with CO_2 Detection*—HCl or HF can volatilize and destroy the gold lining of the infrared cell of a carbon analyzer. To prevent the combustion of collection tubes, as well as cracking of the liner due to temperature changes, the furnace is maintained at ~900°C (1700°F) between short sample runs, except when undergoing maintenance. Follow the manufacturers recommendations for carbon analyzers.

7. Sample Handling

7.1 All Methods require samples free of inorganic carbon, carbonate and undecomposed petroleum-based carbon materials.

7.2 *Method 04.01-A Combustion with CO_2 Detection*—Use material air dried at 37°C, and milled with inerts removed for total organic carbon determinations. If the milled sample is to be stored for more than one month, it should be either stored in a frozen state at -4°C, or dried in a forced-air oven at 70±5°C prior to storage to minimize enzymatic degradation.

Test Method: Organic Carbon. Combustion with CO_2 Detection						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
	04.01-A	04.01-A	04.01-A	04.01-A				04.01-A

04.01-A COMBUSTION WITH CO_2 DETECTION

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Apparatus for Method A

8.1 This method uses a CO_2 analyzer, (e.g. Leco CR-12 carbon analyzer or equivalent).

9. Reagents and Materials for Method A

9.1 *Oxidation Catalysts:*

9.1.1 *Vanadium Oxide (V_2O_5)*—for use with composted materials that contain plastics or are high in volatile fatty acids.

9.1.2 *Aluminum Oxide (Al_2O_3)*—for use with low density composted materials that do not contain plastics and are low in volatile fatty acids.

9.2 *Calibration Standard:*

9.2.1 *Sucrose*—42.1% C, (e.g., Leco brand or equal).

9.2.2 *Calcium Carbonate*—$CaCO_3$.

10. Procedure for Method A

10.1 Review the apparatus instruction manual before proceeding with analyses.

10.2 Set the furnace temperature at 1350°C (~900°C is the normal temperature used for carbon combustion in mineral soils).

10.3 Check and service the anhydrone tubes as required. Refer to the maintenance section of the manual for detailed instructions.

10.4 Condition the instrument with the calcium carbonate calibration standard, (e.g., three or four runs of a 250 mg sample).

10.5 Calibrate the instrument according to the instruction manual. Use the calibration standard appropriate for the expected range of the unknown samples. Use a certified sucrose calibration standard, 42.1% carbon.

10.6 Obtain blank readings with two to three empty combustion boats. Blanks should read less than 0.10%. Higher blanks are an indication of the need for maintenance. Sample values greater than 10% need not be corrected for the blank value.

10.7 Weigh a 0.2 g - 0.5 g compost sample and distribute it evenly across the bottom of the combustion boat. Refer to manufacturer's operation manual for further guidance on sample aliquot size.

10.8 Run the sucrose and calcium carbonate standards with samples. The results should be within 5% of the known value.

10.9 Add approximately 1 cm^3 of vanadium oxide (V_2O_5) to the sample, just enough to cover the compost.

10.10 Insert combustion boat containing sample into furnace.

10.11 Record result.

11. Trouble Shooting for Method A

11.1 For quality control, include a reference sample and two compost standard samples in the analysis. Run one sample in triplicate within each batch of twelve samples. Rerun the calibration standard after each set of ten unknowns followed by the duplication of the first sample in each set of ten. The duplicates should agree within 5-10%.

11.2 Vanadium oxide catalyst (V_2O_5) must be added to the sample to enhance oxidation; organic material in municipal solid waste compost does not combust as readily as that in most other composts or peat. Poor combustion may be associated with the presence of plastics. Low bulk density material such as sphagnum peat burns too rapidly, with the possible loss of sample and inaccurate measurement by the detector because of the rapid release of CO_2. Samples of this nature should be covered with Al_2O_3, (e.g., COM-AID Al_2O_3), that slow the oxidation and give more consistent results. Neither treatment is required for mineral soils.

11.3 The smooth flow of gases through the system is of utmost importance. If it is difficult to obtain consistent results, a complete check of the gas flow is warranted. Give attention to the flow controller, with special attention given to the condition of the check valves.

04.01 METHODS SUMMARY

12. Report

12.1 *Minimum Detectable Concentration*—Compost organic carbon values are reported with three significant figures (±0.1%) and corrected to 70±5°C oven-dry basis.

12.2 Report method and descibe all deviations from the methods provided in TMECC.

13. Precision and Bias

13.1 *Method 04.01-A Combustion with CO_2 Detection*—The precision of this test was examined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

Table 04.01-A1 Organic Carbon, %. Analytical precision within municipal solid waste compost sample (1993) across three sites for two separate sampling periods.

Median	*Std Dev*	*%CV*	*Number of Samples*
27.4	2.6	9.6	10
24.7	2.3	9.1	10
29.4	1.5	5.0	10
30.9	1.6	5.3	10
24.4	0.9	3.8	10
28.6	3.8	13.3	10

NOTE 2A—Coefficient of Variation, %CV = Standard Deviation ÷ Mean × 100.

14. Keywords

14.1 carbon; organic carbon

Method: Nitrogen. Four Methods							**Units:** refer to specific test	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.02-A	04.02-A	04.02-A		04.02-A				04.02-A
04.02-B	04.02-B	04.02-B		04.02-B				04.02-B
		04.02-C		04.02-C				04.02-C
04.02-D	04.02-D	04.02-D		04.02-D				04.02-D

04.02 NITROGEN

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers tests for determinations of nitrogen in compost and compost feedstocks.

1.1.1 *Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique*—This test covers the determination of organic nitrogen with a semi micro-kjeldahl method including the optional nitrate reduction step needed to measure total nitrogen.

1.1.2 *Method 04.02-B Nitrate Nitrogen Determination*—This test covers two common techniques for the determination of nitrate nitrogen.

1.1.3 *Method 04.02-C Ammonium Nitrogen Determination*—This test covers two common techniques for the determination of ammonium nitrogen.

1.1.4 *Method 04.02-D Total Nitrogen by Oxidation.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

Functions of Mineral Nutrients: Macronutrients. pp. 195-268. In Mineral Nutrition of Higher Plants. Marschner, H., ed. Institute of Plant Nutrition. University of Hohenheim, Germany. Academic Press Limited. 1986. 24/28 Oval Road, London NW1 7DX.

Nitrogen Fixation. pp. 173-194. *In* Mineral Nutrition of Higher Plants. Marschner, H., ed. Institute of Plant Nutrition. University of Hohenheim, Germany. Academic Press Limited. 1986. 24/28 Oval Road, London NW1 7DX.

The effects of mineral deficiencies and excesses on growth and composition. *In* Diagnosis of Mineral Disorders in Plants. Vol 1. C. Bould, E.J. Hewitt FRS, and P. Needham, eds. Chemical Publishing, NY. 1984

2.1 *References for Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique:*

AOAC Semiautomated Method No. 976.06 Protein (Crude) in Animal Feed. p 72. *In* Official Methods of Analysis. 1990.

Bowman, D.C., J.L. Paul, and R.M. Carlson. 1988. A method to exclude nitrate from kjeldahl digestion of plant tissues. Commun. in Soil Sci. Plant Anal. 19:205-213.

Bremner, J.M., and C.S. Mulvaney. 1982. Nitrogen-Total. p. 595-624. *In* Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. A.L. Paige, ed. 2nd edition ASA, and SSSA, Madison WI.

Cope, W.C. 1916. Kjeldahl modification for determination of nitrogen in nitro substitution compounds. J. Ind. Eng. Chem. 8:592-593.

Dalal, R.C., K.L. Sahrawat, and R.J.K. Myers. 1984. Inclusion of nitrate and nitrite in the kjeldahl nitrogen determination of soils and plant materials using sodium thiosulfate. Commun. in Soil Sci. Plant Anal. 15:1453-1461.

Stalcup, H. and R.W. Williams. 1955. Volumetric determination of nitrocellulose and nitroguanidine by transnitration of salicylic acid. Anal. Chem. 27:543-546.

Technicon Industrial Method, No. 325-74W Sept. 1974. Ammonical Nitrogen/BD Acid Digests. Technicon Industrial Systems, Tarrytown, NY 10591.

The Kjeldahl Method for Organic Nitrogen. R.G. Bradstreet, ed. Acad. Press, NY and London, 1965.

Wikoff, L. and J.T. Moraghan. 1985. Recovery of soil nitrate by Kjeldahl analysis. Commun. in Soil Sci. Plant Anal. 16:923-929.

2.2 *References for Method 04.02-B Nitrate Nitrogen Determination:*

Gelderman, R.A., and P.E. Fixen. 1988. Recommended Nitrate–N Tests, Chapter 5. *In* Recommended Chemical Soil Test Procedures for the North Central Region. NCR Publication No. 221 (Revised), Bulletin No. 499 (Revised).

Keeney, D.R., and D.W. Nelson. 1982. Inorganic Forms of Nitrogen. *In* Methods of Soil Analysis. Part 2 Chemical and Microbiological Properties. A.L. Page, ed. American Society of Agronomy, Inc. Madison, WI.

RFA Method No. A303-S625-01. 1985. ALPKEM (Perstorp) Corporation. Clackamas, Oregon 97015. Based on US EPA Method 352.2, 1982. Nitrate–Nitrite, Colorimetric, Automated, Cadmium Reduction. *In* Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020.

2.3 *References for Method 04.02-C Ammonium Nitrogen Determination:*

Keeney, D.R., and D.W. Nelson. 1982. Inorganic Forms of Nitrogen. *In* Methods of Soil Analysis. Part 2 Chemical and Microbiological Properties. A.L. Page, ed. American Society of Agronomy. Madison, WI.

RFA Method No. A303-S021. 1986. Ammonium Nitrogen in Water. ALPKEM (Perstorp) Corporation. Clackamas, Oregon 97015. *In* Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020. Ammonia measurement part of Method 351.2, Nitrogen, Kjeldahl, Total", (Colorimetric, Semi-Automated Block Digestor, AAII).

2.4 *References for Method 04.02-D Total Nitrogen by Oxidation:*

Agronomy Monograph no. 9. Chapter 31. Nitrogen—Total. p 595-596. *In* Methods of Soil Analysis, Part 2. Chemical and Microbial Properties. J.M. Bremner and C.S. Mulvaney, ed. 2nd Edition, 1982. ASA-SSSA, 677 S. Segoe Rd., Madison, WI 53711.

3. Terminology

3.1 *ammonia, n*—A colorless, pungent gas, NH_3, extensively used to manufacture fertilizers and a wide variety of nitrogen-containing organic and inorganic chemicals.

3.2 *ammonium, n*—The univalent chemical ion $NH4^+$, derived from ammonia, whose compounds chemically resemble the alkali metals. A microbial and plant nutrient that is a measure of available nitrogen when considered with nitrate nitrogen (NO_3^-–N). Ammonia nitrogen is used for determining the ammonia to nitrate ratio, an indicator of compost maturity.

3.3 *denitrification, n*—To reduce (nitrates or nitrites) to nitrogen-containing gases, through bacterial action. The biological reduction of nitrates and nitrites to molecular nitrogen or oxides of nitrogen. It results in the loss of nitrogen from the soil or compost to the atmosphere.

3.4 *nitrate, n*—The univalent radical NO_3^- or a compound containing it, as a salt or an ester of nitric acid. Nitrate is considered to be a potential pollutant in ground water, surface water, and streams. Low nitrate level in compost indicates immaturity, and will cause ~~soil~~ nitrogen deficiency in plants when the compost is mixed with the soil. Nitrate nitrogen is used for determining the ammonia to nitrate ratio, which is an indicator of compost maturity.

3.5 *nitrification, n*—To oxidize (an ammonia compound) into nitric acid, nitrous acid, or any nitrate or nitrite, especially by the action of nitrobacteria. In composting it refers to the biochemical oxidation of ammonia to nitrate. Aeration enables the conversion of ammonia to nitrate nitrogen, i.e., nitrification.

3.6 *nitrogen, n*—A macronutrient, possibly the most important element required for plant growth. An essential component of proteins, which comprise~~s~~ most of the dry weight of plant and animal cells. It is available to plants in the forms of ammonia (NH_4^+) and nitrate (NO_3^-). A nonmetallic element that constitutes nearly four fifths of the air by volume, occurring as a colorless, odorless, diatomic gas, N_2. It is found in various minerals and in all proteins and is used in a wide variety of important manufactures, including ammonia, nitric acid, TNT, and fertilizers. Atomic number 7; atomic weight 14.0067; melting point (N_2); -209.86°C; boiling point (N_2) -195.8°C; valence 3, 5.

3.7 *organic, n*—Having properties associated with living organisms. Resembling a living organism in organization or development. Organic nitrogen is nitrogen found within organic molecules, which are organic carbon containing molecules.

3.8 *Total Kjeldahl Nitrogen, TKN, n*—The sum of organic nitrogen plus ammonia nitrogen (NH_4^+N). Organic nitrogen has a potential to mineralize to ammonia nitrogen (NH_4^+N). Total Kjeldahl nitrogen should not be used to give the carbon to nitrogen ratio (C:N) of either feedstocks or compost, due to the fact that TKN does not include nitrate nitrogen.

3.9 *Total Nitrogen, TN, n*—In the composting industry total nitrogen has come to mean the sum of Kjeldahl nitrogen plus nitrate nitrogen and nitrite nitrogen. Organic nitrogen plus ammonia nitrogen (NH_4^+) plus nitrate nitrogen (NO_3^-) is used to determine the carbon to nitrogen ratio (%OC:%TKN+NO_3) of feedstock and mature compost. In the strictest sense, it is the sum of Kjeldahl nitrogen plus azide, azine, azo,

hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semi-carbazone.

4. Summary of Test Methods

4.1 *Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique:*

4.1.1 Compost (250 mg) is placed onto nitrogen free paper (e.g., cigarette paper), which is folded to seal the sample. The sample is then digested with 3.5 mL of sulfuric acid at 400°C for 1 to 2 h. A salt/catalyst, Kjeltab® is added to speed up the digestion. When the sample is cool, deionized water is added to dilute the sample. The ammonium formed is measured by an air-segmented continuous flow colorimeter. Ammonium is reacted with sodium salicylate, sodium nitroprusside, and sodium hypochlorite in a buffered alkaline medium. The absorbance of the emerald green complex formed is measured at 660 nm. The concentration of ammonium is determined by reference to a standard calibration curve.

4.1.2 Total Kjeldahl N does not include nitrate or nitrite forms of N, although some conversion of nitrate/nitrite may take place at very high concentrations during the digest. To quantitatively include these forms in the Kjeldahl procedure for total N, the nitrate reduction step must be included in the digest step (Method 04.02-A, step 10.2). The nitrate reduction step does not impact the total N value when the concentration of NO_3-N + NO_2-N is less than 500 mg kg^{-1}.

4.1.2.1 *Nitrate Reduction Step*—Salicylic acid is placed onto a separate cigarette paper and added with the sample and sulfuric acid. This mixture is left overnight. Sodium thiosulfate is added to the sample mixture the next morning. The sample is ramped to 320°C until frothing ceases and then cooled to 240°C after which the salt/catalyst Kjeltab® is added and the sample digested at 400°C for 1 to 2 h. When the sample is cool, deionized water is added to dilute the sample. Ammonium formed is measured by an air-segmented continuous flow colorimeter. Ammonium is reacted with sodium salicylate, sodium nitroprusside, and sodium hypochlorite in a buffered alkaline medium. The absorbance of the emerald green complex formed is measured at 660 nm. The concentration of ammonium is determined by reference to a standard calibration curve.

4.1.3 An alternative way of measuring total nitrogen is to use a Dumas-type nitrogen analyzer, which are available from several manufacturers. The Dumas-type analyzer measures all forms of nitrogen in the measurement of nitrogen gas.

4.2 *Method 04.02-B Nitrate Nitrogen Determination*—Nitrate nitrogen is determined by ion chromatography on compost extracts or by colorimetry on clear sample extracts.

4.2.1 *Nitrate by Ion Chromatography*—The nitrate ion displays strong absorbance in the lower range of UV. Refer to the operator's manual for wavelength.

4.2.2 *Nitrate by Colorimetry*—Nitrate in compost is determined by reducing nitrate to nitrite in a copperized cadmium reductor. The nitrite ion then reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthyl ethylene diamine dihydrochloride to form a reddish-purple azo dye. The absorbance of nitrate–N is measured at 540 nm. This value also includes the nitrite–N form.

4.3 *Method 04.02-C Ammonium Nitrogen Determination:*

4.3.1 *Ammonium by Ion-Selective Electrode*—This technique uses a hydrophobic gas-permeable memberane to separate the sample solution from a solution of ammonium chloride. The pH of the sample solution is raised to 11 with a strong base. The aqueous ammonia diffuses through the permeable memberane where the pH change is monitored. Measurements are made with a pH meter or an ion-specific meter.

4.3.2 *Ammonium by Colorimitry*—This technique involves a reaction of ammonia with salicylate, nitroprusside and hypochlorite in a buffered alkaline medium. The absorbance of the emerald green complex formed is measured at 660 nm. Ammonia salts are water soluble, ammonium is generally held by ion exchange and requires a high concentration of salt for its complete removal; 2*M* KCl is most commonly used to exchange the ammonium ion with the potassium ion.

4.4 *Method 04.02-D Total Nitrogen by Oxidation:*

4.4.1 *Wet Oxidation Technique*—Refer to Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique:

4.4.1.1 Include nitrate reduction steps outlined in Method 04.02-A, or

4.4.1.2 Sum %N from TKN determination without nitrate reduction step and %N from NO_3^-–N determined using *Method 04.02-B Nitrate Nitrogen Determination by Colorimetry.*

4.4.2 *Oxidation by Combustion or Dry Oxidation Technique*—A sample is heated with CuO at high temperature (> 600°C) in a stream of purified CO_2, and the gases liberated are led over hot Cu to reduce nitrogen oxides (mainly N_2O) to N_2, and then over CuO to convert CO to CO_2. The N_2-CO_2 mixture obtained is collected in a nitrometer containing concentrated alkali that absorbs the CO_2, and the volume of N_2 gas is measured. The method of measuring the N_2 may vary with instrument.

5. Significance and Use

5.1 Nitrogen is a macronutrient and may be the most important element required for plant growth. Nitrogen is an essential part of proteins, which comprise most of the dry weight of plant and animal cells. It is available to plants in the forms of ammonia (NH_4^+) and nitrate (NO_3^-). Nitrification in composting refers to the biochemical oxidation of ammonia to nitrate and requires aeration. Denitrification refers to the biological reduction of nitrate to molecular nitrogen or oxides of nitrogen and occurs in aneorobic pockets or aggregates within compost. Dinitrogen and the various oxides are volatile at ambient laboratory temperature and readily lost to the atmosphere.

5.1.1 Nitrate nitrogen (NO_3^-–N) is a soluble form of nitrogen that readily leaches from soil. The US EPA considers nitrate to be a potential pollutant in ground water, surface water, and streams. Low nitrate levels in conjunction with high ammonium concentrations in compost indicate instability and potentially high microbial activity that may cause nitrogen deficiency in plants when applied to a nitrogen deficient soil. Nitrate nitrogen is used for determining the ammonia to nitrate ratio, an indicator of compost stability status.

5.2 *Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique:*

5.2.1 This method also applies to soil, peat, plants, manure, foods, feeds and other biological material. This method is a semi-micro adaptation of the AOAC 976.06 method, Protein (Crude) in Animal Feed - Semi-automated Method. The difference is that reagents, sample size, and digestion tube size are all reduced five-fold from the AOAC method, and selenium is substituted for mercury as the catalyst.

5.2.2 The Kjeldahl conditions are generally not successful in converting N–N or N-O bonds to NH_4^+, and the N in organic compounds that contain these bonds are not measured. In addition, a fraction of nitrate in samples will also be reduced, presumably because of the reducing power of the organic matter. Nitrate can be quantitatively included when reduced to the NH_4^+ form by pre-treating samples with salicylic acid and sodium thiosulfate, or excluded by adding hydrogen peroxide to oxidize the organic matter.

5.2.3 In the salicylic acid method, the nitrates react with the salicylic acid in an acid environment to form nitro compounds, which are reduced to the corresponding amino compounds by heating the mixture with sodium thiosulfate. The identity of the nitro compounds formed in the procedure has not been fully established, but work indicates that the main product of nitration is 5-nitrosalicylic acid, along with small amounts of 3-nitrosalicylic acid. Water can block the nitration process, so samples must be dry before treatment (air-dried at 36°C or oven-dried at 70±5°C).

5.3 *Method 04.02-B Nitrate Nitrogen Determination:*

5.3.1 Nitrate nitrogen (NO_3^-–N) is a water soluble, plant available and leachable from of nitrogen. Nitrate is considered to be a potential pollutant in ground water, surface water, and streams. Low nitrate levels in compost indicate immaturity. Nitrate nitrogen is used for determining the ammonia to nitrate ratio, an indicator of compost maturity.

5.3.2 Nitrate (NO_3) is unstable and subject to rapid changes through ammonification, nitrification, and gaseous losses following sample collection. This is controlled primarily by microbial activity. Microbial activity is minimized when compost samples are stored at a temperature between -4°C and 4°C. Samples should be transported at this cold temperature, and prepared for analysis at as-received moisture upon receipt. If sample preparation cannot be initiated upon arrival the samples should be stored at temperatures between -4°C and 4°C.

5.3.3 Extract can be stored for up to five days at 4°C before analysis. For longer periods, the extract must be acidified to a pH slightly less than 2.0 using HCl and reneutralized before ammonium determination.

5.4 *Method 04.02-C Ammonium Nitrogen Determination:*

5.4.1 Ammonium (NH_4) is unstable and subject to rapid changes through ammonification, nitrification, and gaseous losses following sample collection. As with nitrates, this is controlled primarily by microbial activity. Microbial activity is minimized when samples are stored at a temperature between -4°C and 4°C. Samples should be transported at this cool temperature, and prepared for analysis upon receipt. If sample preparation cannot be initiated upon arrival the samples should be stored at temperatures between -4°C and 4°C.

5.4.2 Extract can be stored for up to five days at 4°C before analysis. For longer periods, the extract must be acidified to a pH slightly less than 2.0 using HCl.

5.5 *Method 04.02-D Total Nitrogen by Oxidation*—In the composting industry total nitrogen has come to mean the sum of organic nitrogen (Kjeldahl) plus nitrate and nitrite. Organic nitrogen plus ammonia nitrogen (NH_4^+–N) plus nitrate nitrogen (NO_3^-–N) is used to determine the carbon to nitrogen ratio (C:N) of compostable feedstock and mature compost. In the strictest sense, it is the sum of organic nitrogen plus azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime, and semi-carbazone.

6. Interference and Limitations

6.1 *Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique*—The forms of nitrogen

measured are those that can be reduced to ammonium (NH_4^+) when the sample is digested in hot sulfuric acid. This enhances the complete recovery and rate of conversion of organic nitrogen to NH_3

6.1.1 Temperature strongly influences the amount of salt needed for the Kjeldahl digest. High concentrations of K_2SO_4 might cause the digest to solidify upon cooling. Careful attention must be given to mixing the warm digest with water after the digestion process.

6.1.2 Ammonia loss through volatilization during the air-drying step of sample preparation may result in a significantly lower total nitrogen determination for some feedstock and raw compost samples, (e.g., animal manure).

6.2 *Method 04.02-B Nitrate Nitrogen Determination:*

6.2.1 *Nitrate Nitrogen by Ion Chromatography*—Chloride and sulfate at high concentrations can interfere with nitrate detection. Additional sample dilution will diminish their interferences. Further dilution will deteriorate the nitrate nitrogen detection limit. Temperature compensation is necessary. Measurements are sensetitive to temperature fluctuations.

6.2.2 *Nitrate Nitrogen by Colorimetry*—The sample extract must be clear for accurate measurements. Suspended particles cloud a sample and inflate nitrate readings. Colored sample extracts will inflate nitrate readings if proper adjustments are not made. If a sample is yellow, brown or anything other than clear, results will not be reliable.

6.3 *Method 04.02-C Ammonium Nitrogen Determination:*

6.3.1 *Ammonium by Ion-Selective Electrode*—The presence of volatile amines will inflate readings. Methylamine and ethylamine are detected as NH_3. Mercury (Hg^{2+}), which forms a complex with NH_3 under alkaline conditions, is also detected as NH_3.

6.3.2 *Ammonium by Colorimetry*—The sample extract must be clear for accurate measurements. Suspended particles cloud a sample and inflate nitrate readings. Colored sample extracts will also inflate nitrate readings. If a sample is yellow, brown or anything other than clear, results will not be reliable.

6.4 *Method 04.02-D Total Nitrogen by Oxidation*—Complete recovery of fixed NH_4^+–N may not occur with some materials containing high levels of fixed N.

7. Sample Handling

7.1 *Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique*:

7.1.1 *Homogenous, immature compost and feedstock high in volatile N*—Use as-received moist material. Gently rub the sample through a 4-mm sieve and thouroughly mix the sieved material. Determine sample moisture on a parallel aliquot. Avoid cross contamination of volatile NH_4^+ among samples.

7.1.2 *Heterogeneous and coarse compost low in volatile N*—Use air-dried material that is finely milled to a powder with inerts removed (see sample handling section). Store samples in separate sealed containers at ambient laboratory temperature for no more than two or three weeks. Store material in a frozen state (-4°C) after three weeks to minimize enzymatic degradation of the sample.

7.2 *Method 04.02-B Nitrate Nitrogen Determination*—Compost should be moist as received (40-60% moisture, wet weight basis). Analysis must be performed upon sample receipt. If any delay in analysis is anticipated, sample should be refrigerated at 4°C for no more than one week.

7.3 *Method 04.02-C Ammonium Nitrogen Determination*—Compost should be moist as received (40-60% moisture, wet weight basis). Analysis must be performed upon sample receipt. If any delay in analysis is anticipated, sample should be refrigerated at 4°C for no more than one week.

7.3.1 Ammonium (NH_4) is unstable and subject to rapid oxidation to nitrate and to gaseous losses following sample collection. This instability is a function of microbial activity. Microbial activity is minimized at temperatures below 4°C. Samples should be transported at this cool temperature, and prepared for analysis upon receipt. If sample preparation cannot be initiated upon arrival, the samples should be stored at temperatures near 4°C or frozen to inhibit microbial activity.

7.3.2 Samples should be transported and stored in sealed containers to minimize cross contamination of separate samples. Also, air drying samples in open containers will absorb and/or release ammonium, contaminating the space with nitrogen. Analysis of air dried samples prepared under open conditions should be avoided.

7.4 *Method 04.02-D Total Nitrogen by Oxidation:*

7.4.1 *Homogenous, immature compost and feedstock high in volatile N*—Use as-received moist material. Gently rub the sample through a 4-mm sieve and thouroughly mix sieved sample material. Determine sample moisture on a parallel aliquot. Avoid cross contamination of volatile NH_4^+ among samples.

7.4.2 Ammonia loss through volatilization during the air-drying step of sample preparation may result in a significantly lower total nitrogen determination for some samples.

7.4.3 *Heterogeneous and coarse compost low in volatile N*—Use air-dried material that is finely milled to a powder with inerts removed. Store samples in separate sealed containers at ambient laboratory temperature for no more than two or three weeks. Store material in a frozen state (-4°C) after three weeks to minimize enzymatic degradation of the sample.

Test Method: Nitrogen. Total Kjeldahl Nitrogen							**Units:** % dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
04.02-A	04.02-A	04.02-A		04.02-A				04.02-A

04.02-A TOTAL KJELDAHL NITROGEN, SEMI-MICRO KJELDAHL TECHNIQUE

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

COMMENT—This method reflects laboratory practices for TKN and TN determinations at the Research Analytical Laboratory, Department of Soil, Water, and Climate; U of MN, St. Paul, MN 55108; by Robert Munter.

8. Apparatus for Method A

8.1 *Aluminum Digestion Block*—64 hole, custom built.

8.2 *Pyrex Glass Tubes for Digestions*—25 × 200 mm, with 50 mL graduation mark (e.g., Folin-Wu type, Corning no. 7900 or equivalent).

8.3 *AutoAnalyzer*—automated apparatus, (e.g., Technicon AutoAnalyzer II with ammonium analytical cartridge, Technicon Corp).

9. Reagents and Materials for Method A

9.1 *Concentrated Sulfuric Acid (H_2SO_4).*

9.2 *Tecator Kjeltabs®*—Tablets that consist of 1.5 g K_2SO_4 and 7.5 mg Se (e.g., No. 13 159-C, Fisher Scientific).

9.3 *Salicylic Acid ($HC_7H_5O_3$).*

9.4 *Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$).*

9.5 *Stock Nitrogen Standard*—1000 mg L^{-1} N. Weigh 2.3584 g of dried Ammonium Sulfate $(NH_4)_2SO_4$ in a 500-mL volumetric flask and dilute to volume with deionized water. Working standards are made up in a composite of digested blanks diluted to the 50 mL mark of the digestion tubes at concentrations that cover the range found in the samples.

9.6 *Stock Sodium Hydroxide (NaOH) Solution (20 %)*—Make the solution of Stock 50 % (w v^{-1}) Sodium Hydroxide solution by weighing 250 g of NaOH pellets into a 500 mL volumetric flask. Dilute to volume with deionized water. To 600 mL of deionized water, add 400 mL of 50 % w v^{-1} sodium hydroxide solution. Cool to ambient laboratory temperature and dilute to 1 L with deionized water.

9.7 *Stock 20 % Solution of Sodium Potassium Tartrate ($NaKC_4H_4O_6 \cdot 4H_2O$)*—Dissolve 200 g of sodium potassium tartrate in about 600 mL of deionized water and bring to volume in a 1 L volumetric flask, mixing thoroughly.

9.8 *Stock Buffer Solution 0.5 M Sodium Phosphate (Na_2HPO_4)*—Dissolve 134 g of sodium phosphate, dibasic, crystal (or 71 g of sodium phosphate, dibasic, anhydrous) in about 800 mL of deionized water. Add 40 g of sodium hydroxide solution, 50 % w v^{-1}, dilute to 1 L with distilled water and mix thoroughly.

9.9 *Working Buffer Solution*—Combine the reagents in this order: add 250 mL of stock 20 % sodium potassium tartrate solution to 200 mL of stock 0.5 *M* buffer solution while swirling. Continuing to swirl, add 250 mL of 20 % sodium hydroxide solution. Dilute to 1 L with deionized water then add 1.0 mL Brij-35 (surfactant, 30 % solution 20-25 drops) and mix thoroughly.

9.10 *Sulfuric Acid/Sodium Chloride (H_2SO_4/NaCl) Solution*—Dissolve 100 g of sodium chloride in about 600 mL of deionized water. Add 7.5 mL of sulfuric acid and dilute to 1 L with deionized water. Add 1.0 mL of Brij-35 (about 20 drops) and mix thoroughly.

9.11 *Sodium/Salicylate-Sodium/Nitroprusside $[NaC_7H_5O_3]/[Na_2Fe(CN)_5 \cdot NO \cdot 2H_2O]$ Solution*—Dissolve 150 g of sodium salicylate and 0.30 g of sodium nitroprusside in about 600 mL of distilled water. Filter through fast filter paper into a 1 L volumetric flask and dilute to volume with distilled water. Add 1.0 mL of Brij-35 and mix thoroughly. Store in a light resistant container.

9.12 *Sodium Hypochlorite Solution, 0.315%*—Dilute 6.0 mL of sodium hypochlorite solution to 100 mL with distilled water. Add 0.1 mL (2 drops) of Brij-35 and mix thoroughly. Prepare fresh daily. Any commercial bleach solution containing 5.25% available chlorine is satisfactory.

9.13 *Sample Wash Solution*—Triple deionized water without a wetting agent.

10. Procedure for Method A

10.1 *Digestion:*

10.1.1 Analytically transfer a 150 mg – 250 mg aliquot of compost onto nitrogen-free cigarette paper, (e.g., OCB from R. J. Reynolds Tobacco Company). Very low

bulk density (fluffy) material requires larger sized paper, (e.g., Zig-Zag™ from United States Tobacco Co.). Fold paper to keep material within paper.

NOTE A1—Results are adjusted to a 70±5°C oven dry basis.

NOTE A2—Refer to step 10.2 for the Nitrate Reduction Step.

10.1.2 *Digestion Batch Set-up*—For each digestion block, 64 tube capacity, include the following:

10.1.2.1 Three tubes consisting of blanks comprised of weighing paper, standard amount of acid, and one salt/catalyst tablet (Kjeltab®).

10.1.2.2 Duplicate every eight samples.

10.1.2.3 Include a minimum of one reference sample with each batch of 8 to 48 samples.

10.1.2.4 Include one NIST pine needles reference sample or other appropriate NIST reference sample periodically. If a new batch of "in-house" reference material is to be included with samples, the reference should be included with every 8 to 24-samples until a mean with low variability is established for the new check.

10.1.3 Drop the folded paper containing the sample into a clean, dry digestion tube and add 3.5 mL of concentrated sulfuric acid. Swirl gently to mix.

NOTE A3—If nitrate is to be reduced and included in the result for reporting *Total–N*, go to *step 10.2* before continuing to *step 10.1.4* If *Total–N* is achieved by a separate determination for nitrate, then continue to *10.1.4*.

10.1.4 Add one digestion tablet (Kjeltab®).

NOTE A4—Record the container number or lot number of each new can of digestion salt/catalyst tablets that is opened for blanks.

10.1.5 Place the tubes into a preheated (400°C setting) aluminum heating block. The temperature of the digest must be within 360 - 410°C.

10.1.6 Digest the samples for ½ h after clearing (i.e., disappearance of carbon). MSW-compost samples generally take between 1 to 2 h. to clear.

10.1.7 Remove the tubes from the heating block and allow them to cool for about 10 min.

10.1.8 Carefully add 10 to 20 mL of deionized water to the digestion tubes while they are still warm. Mix with a vortex mixer to dissolve any crystals that may have formed. Avoid adding water when the tubes are too hot to avoid spattering and possible sample loss and injury.

10.1.9 Dilute to the 50 mL mark with deionized water and mix after capping with 5 mL disposable beakers.

10.1.10 Analyze for ammonium. Go to Step 10.3

10.2 *Optional Nitrate Reduction Step for Total Nitrogen:*

10.2.1 Weigh 75 mg of salicylic acid into a separate cigarette paper and add along with the sample to the H_2SO_4. Let the sample stand for 1 h, then swirl until thoroughly mixed and cigarette paper is dissolved.

10.2.2 Let the sample stand for 12 h – 18 h.

10.2.3 Weigh 500 mg of sodium thiosulfate and add to each tube. The use of a funnel will help direct the chemical to the bottom of the tube.

10.2.4 Heat the mixture in the digestion block slowly by ramping the digestion block temperature to 320°C and until frothing ceases.

10.2.5 Remove from the block and cool samples to approximately 240°C.

10.2.6 Return to step 10.14 above.

10.3 *Ammonium Determination:*

10.3.1 Remove reagents from the refrigerator and warm to ambient laboratory temperature. Prepare fresh hypochlorite solution. Prepare the colorimeter for use.

10.3.2 Pour samples into 5-mL plastic sampler cups, rinsing each cup with a small amount of the sample digest before filling.

10.3.3 Attach the air lines of the ammonium channel to an air scrubber containing 10% sulfuric acid (v v^{-1}).

10.3.4 Pump deionized water through the manifold tubing (lines) for 5 to 10 min. Place all reagent lines in the their respective reagent bottles with the exception of the sodium salicylate line. Place the lines in the arrangement as described in the method.

10.3.5 When the reagents have been pumping for at least 5 min, place the salicylate line in its container and allow the system to equilibrate for 10 min.

CAUTION !—If a precipitate appears after the addition of sodium salicylate, immediately stop the proportioning pump and flush the coils with water using a syringe. Precipitation of salicylic acid occurs at low pH. Before restarting the system, check the concentration of the sulfuric acid solution and/or the working buffer solution.

10.3.6 The first sample cups in a run include five calibrating standards followed by a blank. The calibrating standards consist of five digested blanks containing acid and a digestion salt tablet. After digestion, an appropriate amount of ammonium sulfate standard solution is added to each of the tubes and all tubes are diluted to the 50-mL mark. Include a blank and the highest calibration standard every 32 samples as a drift control. Each sample run also ends with this drift control set. The computer software (e.g., Labtronics Inc.; 95 Crimea Street; Guelph, Ontario, Canada) performs drift correction as required. Include a duplicate sample every 15 to 20 samples.

10.3.7 After each run, remove the sodium salicylate line first and allow to pump for 5 min until the reagent

has cleared the line. Then remove the other lines and place in deionized water. Run the pump for several min on high and then 10 min on low to clear the lines.

NOTE A5—The ratio of acid to salt (H_2SO_4:K_2SO_4) is important. This ratio is referred to as the salt index and should be between 0.88 and 1.5. The amount of residual acid remaining after digestion should be 1.3 and 2.1 mL, respectively for these salt ratios. Lower salt indices cause higher oxidation temperatures and shorter digestion times.

NOTE A6—The catalyst used in the AOAC method is mercury (AOAC, 1990). The procedure shown here is modified to use selenium. The use of mercury or selenium creates a hazardous waste disposal problem and proper disposal of the wastes is necessary.

Test Method: Nitrogen. Nitrate Nitrogen Determination						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
04.02-B	04.02-B	04.02-B		04.02-B				04.02-B

04.02-B NITRATE NITROGEN DETERMINATION

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

11. Apparatus for Method B

11.1 *Extraction Flasks*—250-mL hard plastic or glass capped flasks, 1-L Ziploc® plastic bags, etc.

11.2 *Shaker*—wrist or table, capable of operating at 180 reciprocations or excursions per minute.

11.3 *Detection Apparatus*

11.3.1 *Ion Chromatograph*—with anion column, (e.g., Dionex, DX-120), or

11.3.2 *Colorimeter*—configured with nitrate channels fitted with wavelength filter for nitrate/nitrite, 540 nm, (e.g., RFA-300, Alpkem Rapid Flow Analyzer).

12. Reagents and Materials for Method B

12.1 *Ion Chromatography:*

12.1.1 *Extracting Solution (H_2O)*—17 MΩ·cm, Type-II minimum standard deionized, ammonia-free water.

12.2 *Colorimetry by Cadmium Reduction:*

12.2.1 *Extracting Solution (2M KCl)*—Dissolve 1491 g reagent grade KCl in 10 L of 17 MΩ deionized water.

12.2.2 *Stock Imidazole Buffer Solution (0.1M Imidazole)*—Dissolve 6.81 g Imidazole in 800 mL deionized water. Adjust pH to 7.5 with concentrated HCl. Add more water to make a final volume of 1 L.

12.2.3 *Copper sulfate (0.01M)*. Dissolve 2.5 g copper sulfate in 800 mL deionized water. Add more water to make a final volume of 1 L.

12.2.4 *Working Imidazole Buffer Solution (0.05M Imidazole)*—Add 625 µL of 0.01*M* $CuSO_4$ to 125 mL of Stock Imidazole (0.1*M*) and dilute to 250 mL with deionized water. Add 0.25 mL Brij-35 (30%). Prepare fresh daily.

12.2.5 *Open Tubular Cadmium Reactor Coil*—24 in. Perstorp Corporation no. A303-0500-24.

12.2.6 *Color Developing Solutions*—(SAN) Sulfanilimide Solution: dissolve 10 g sulfanilimide in 600 mL deionized water containing 100 mL concentrated HCl. Mix and dilute to 1 L volume. Add 0.5 mL Brij-35 (30%) and mix gently to avoid foaming. Store at approximately 4°C in a sealed, plastic container. (NED) *N*-(1-naphthyl) ethylenediamine dihydrochloride: Dissolve 1.0 g *N*-(1-naphthyl) ethylenediamine dihydrochloride in 800 mL deionized water. Add 0.5 mL Brij-35 (30%). Add more water to make a final volume of 1 L . Store at 2 to 4°C in a plastic container.

12.2.7 *Activation Procedure for the Tubular Cadmium Reactor Column. See Alpkem (Perstorp) method referenced*—Fill a 50-mL beaker with equal volumes of STOCK Imidazole buffer and copper sulfate solution. Sleeve a 3 in length of 0.034 in polyethylene tubing to each end of the reactor. Use 0.035 in tubing to sleeve the polyethylene. Use 0.081 in tubing to sleeve the 0.035 in tubing. Fill a syringe with the buffered copper solution so that no air bubbles remain. Attach the syringe to one end of the polyethylene tubing. Push the buffered copper solution into the reactor, taking care not to introduce bubbles into the reactor. Repeat 3 to 4 times.

12.2.7.1 Using the syringe, rinse and fill the reactor with STOCK Imidazole buffer before installing on the manifold. Do not leave the buffered copper solution in the reactor after the activation treatment. When the reactor is not in use, remove it from the manifold, fill with STOCK Imidazole buffer and seal by joining the ends with a short length of transmission tubing. Reactor efficiency should be verified by comparing a nitrite–N standard to a nitrate–N standard at the same concentration. It may be necessary to stabilize the reactor after activation. Aspirate a 1 mg L^{1} standard continuously through the sample line for 10 min, then allow the system to pump reagents for 20 min.

12.3 *Nitrate Calibration Solution Standard*—Stock Standard Solution 1000 mg L^{-1} NO_3^- (225.8 mg L^{-1} NO_3^-–N). Weigh 1.6305 g of oven dry (105°C) KNO_3 into a 1-L volumetric flask. Make up to volume with extracting solution. Working Standards: 5.0 to 200 mg L^{1} NO_3^- (1.13 to 45.2 mg L^{-1} as NO_3^-–N) in solution. For example, a sample with a moisture content of 50% (wet basis), the range is equivalent to 12.4 to 497 mg kg^{-1} of NO_3^-–N sample on a dry weight basis.

13. Procedure for Method B

13.1 *Prepare Samples:*

13.1.1 *Compost Aliquot Moisture*—Determine the total solids ratio and moisture content on a parallel aliquot of the sample.

13.1.1.1 *Option One*—Measure and record the as-received tare weight of the aliquot. Oven dry the aliquot in a microwave oven with high temperature setting for approximately 5 min, or until sample weight-change diminishes to nill. Calculate the total solids ratio by dividing the microwave oven dry weight by the as-received moist weight.

CAUTION—Metal fragments, i.e., inert contaminants, in the compost aliquot can cause the sample to ignite or char while inside the microwave oven.

13.1.1.2 *Option Two*—If no microwave oven is available, follow the protocols to determine total solids as described in Method 03.09 Total Solids and Moisture; the complete procedure is required for reporting sample moisture content. Method 04.02-B must be performed after the determination of total solids and moisture.

13.1.2 Weigh 40.0 g dry-weight equivalent of as-received moist compost (Equation 13.1.2.1) into the sample container, (e.g., 250-mL capped flask).

13.1.2.1 Determine the dry-weight equivalent aliquot size.

$$A = B \div [C \times 0.01] \qquad \textit{Equation 13.1.2.1}$$

where:

A = mass of as-received moist compost aliquot, g
B = dry-weight equivalent of sample, i.e., 40.0 g,
C = sample total solids content, % wet weight basis, and
0.01 = factor to convert from percentage to fraction, unitless.

13.1.3 Bring the liquid fraction of the 1:5 solids:liquid slurry to an equivalent of 200 mL by adding deionized water to the as-received moist compost aliquot (Equation 13.1.3.1). This step is based upon an assumption that 1 mL is equivalent to 1 g of the as-received compost liquid fraction, and that 1 mL of water is equivalent to 1 g of water.

13.1.3.1 Determine the required volume of extractant.

$$A = B - [C - 40] \qquad \textit{Equation 13.1.3.1}$$

where:

A = volume of extractant required, mL,
B = target 1:5 slurry liquid fraction, 200 mL,
C = mass of as-received compost aliquot, g, and
40 = total solids fraction of the as-received compost aliquot, g.

13.1.4 Place the 1:5 slurry on a shaker for 20 min at 180 reciprocations or excursions per minute.

13.1.5 Maintain slurry at ambient laboratory temperature, (e.g., 20-23°C).

13.1.6 Extract the 1:5 Slurry liquid fraction.

13.1.6.1 Transfer the slurry to a 200-mL centrifuge tube. Centrifuge at 8000 *g* for fifteen min to separate solid and liquid fractions.

13.2 *Ion Chromatography (Preferred Technique)*

13.2.1 Determine the nitrate present in centrifugate.

13.3 *Colorimetry by Cadmium Reduction (not generally recommended for use with stained extracts)*.

13.3.1 Determine the nitrate present in centrifugate.

NOTE 1B—If the extract cannot be analyzed within 4 h, place the sample in 4°C refrigerated store for no more than five days. Acidify to pH 2.0 with HCl if storage exceeds five days.

14. Calculations and Corrections for Method B

14.1 Data is collected as mg L^{1} (ppm) of NO_3^- in solution, the results are converted to elemental N (NO_3^-–N) for reporting purposes.

14.2 *Data and Reporting Units*—NO_3^-–N mg kg^{-1} Compost on an oven dried basis (70±5°C).

14.3 Calculate concentration of nitrate in elemental form per kg of compost:

$$A = B \times [C + (D - E)] \div [E \times 4.429] \qquad \textit{Equation 14.2.2}$$

ASSUME—1 mL ≡ 1 g; and 1 mg L^{-1} ≡ 1 mg kg^{-1}

where:

A = elemental N as NO_3^- per kg of compost, mg kg^{-1}.
B = measured concentration of NO_3^- (mg L^{-1}),
C = volume of extraction solution, mL, and
D = mass of the sample aliquot at as as-received moisture, g,
E = Oven dry weight at 70±5°C g,
4.429 = factor to convert NO_3^- to elemental N (NO_3^-–N).

Test Method: Nitrogen. Ammonium Nitrogen Determination							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
		04.02-C		04.02-C				04.02-C

04.02-C AMMONIUM NITROGEN DETERMINATION

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

15. Apparatus for Method C

15.1 *Extraction Flasks*—250-mL hard plastic or glass capped flasks, 1-L Ziploc® plastic bags, etc.

15.2 *Shaker*—wrist or table, capable of operating at 180 reciprocations or excursions per minute.

15.3 *Detection Apparatus (three options are presented)*

15.3.1 *Ion-Selective Electrode*

15.3.1.1 *Electrometer*—pH meter with resolution of 0.1 mV and range of –700mV to +700mV, or equivalent meter.

15.3.1.2 *Magnetic Stirrer*—TFE-coated stirring bar.

15.3.1.3 *Ammonia Ion-Selective Electrode.*

15.3.2 *Colorimeter*—configured with ammonia channel fitted with wavelength filter for ammonium, 660 nm (e.g., RFA-300, Alpkem Rapid Flow Analyzer).

16. Reagents and Materials for Method C

16.1 *Ion-Selective Electrode:*

16.1.1 *Extracting Solution (H_2O)*—17 MΩ·cm deionized, ammonia-free water.

16.2 *Colorimetry:*

16.2.1 *Extracting Solution (2M KCl)*—Dissolve 1491 g reagent grade KCl in 10 L of 17 MΩ deionized water.

16.2.2 *Sodium Hydroxide (NaOH), 5M (stock) Solution*—Dissolve 200 g NaOH in 700 mL deionized water. Cool and dilute to 1 L. This dilution creates a lot of excess heat. Add the NaOH cautiously with frequent stirring.

16.2.3 *Sodium Potassium Tartrate ($KNaC_4H_4O$ 6.4·H_2O)*—Dissolve 200 g of sodium potassium tartrate in 700 mL of deionized water. Dilute to 1 L.

16.2.4 *Stock Buffer Solution, Sodium Phosphate-Dibasic (Na_2HPO_4)*—Dissolve 134 g of sodium phosphate-dibasic in approximately 500 mL of deionized water in a 1 L volumetric flask. Add 100 mL of stock 5*N* NaOH to the sodium phosphate stock buffer solution. Mix and dilute to 1 L with deionized water.

16.2.5 *Working Buffer Solution*—Under continuous stirring, add 62.5 mL of stock sodium potassium tartrate solution to 50 mL of stock buffer (Na_2HPO_4) in a 250 mL volumetric flask. Add 7.5 mL of 5*N* NaOH while stirring. Dilute to 250 mL with deionized water and mix well. Add 0.25 mL of Brij-35 (30%) and mix very gently so as not to create foam. Prepare solution weekly.

16.2.6 *Sodium Salicylate and Sodium Nitroferricyanide Solution ($NaC_7H_5O_3$ and $Na_2Fe(CN)_5NO·2H_2O$)*—Dissolve 75 g of sodium salicylate and 0.15 g of sodium nitroferricyanide in 400 mL of deionized water, mix and dilute to 500 mL. Filter the solution through No. 2 filter paper into a 500 mL in a brown bottle.

16.2.7 *Sodium Hypochlorite Solution (NaOCl)*—Dilute 6 mL of 5.25 % sodium hypochlorite solution (household bleach) to 100 mL with deionized water. Prepare solution weekly.

16.3 *Ammonium Chloride Calibration Standards (NH_4Cl)*—Stock Solution of 200 mg L^{-1} NH_4^+ (156 ppm NH_4^+–N). Dissolve 296.9 mg of dried (105°C) ammonium chloride in 500 mL of deionized water.

16.3.1 *Working standard solutions*—1.0 to 15 mg L^{-1} NH_4^+ (or 0.78 to 11.7 mg L^{-1} as NH_4^+–N). For a sample with a moisture content of 50%, this range is equivalent to 8.58 to 128.7 mg kg^{-1} as NH_4^+–N in the sample on a dry weight basis.

17. Procedure for Method C

17.1 *Prepare Samples:*

17.1.1 *Compost Aliquot Moisture*—Determine the total solids ratio and moisture content on a parallel compost sample aliquot.

17.1.1.1 *Option One*—Measure and record the as-received tare weight of the aliquot. Oven dry the aliquot in a microwave oven with high temperature setting for approximately 5 min, or until sample weight-change diminishes to nill. Calculate the total solids ratio by dividing the microwave oven dry weight by the as-received moist weight.

CAUTION—Metal fragments, i.e., inert contaminants, in the compost aliquot can cause the sample to ignite or char while inside the microwave oven.

17.1.1.2 *Option Two*—If no microwave oven is available, follow the protocols to determine total solids as described in Method 03.09 Total Solids and Moisture; the complete procedure is required for reporting sample moisture content. Method 04.02-C must be performed after the determination of total solids and moisture.

17.1.2 Weigh 40.0 g dry-weight equivalent of as-received moist compost (Equation 17.1.2.1) into the sample container, (e.g., 1-qt sized Ziploc®-type plastic bag or 500-mL screw-cap Erlenmeyer flask).

17.1.2.1 Determine the dry-weight equivalent aliquot size.

$$A = B \div [C \times 0.01] \qquad \textit{Equation 17.1.2.1}$$

where:

A = mass of as-received moist compost aliquot, g
B = dry-weight equivalent of sample, i.e., 40.0 g,
C = sample total solids content, % wet weight basis, and
0.01 = factor to convert from percentage to fraction, unitless.

17.1.3 Bring the liquid fraction of the 1:5 solids:liquid slurry to an equivalent of 200 mL by adding deionized water to the as-received moist compost aliquot (refer to Equation 17.1.3.1). This step is based upon an assumption that 1 mL is equivalent to 1 g of the as-received compost liquid fraction, and that 1 mL of water is equivalent to 1 g of water.

17.1.3.1 Determine the required volume of extractant.

$$A = B - [C - 40] \qquad \textit{Equation 17.1.3.1}$$

where:

A = volume of extractant required, mL
B = target 1:5 slurry liquid fraction, 200 mL
C = mass of as-received compost aliquot, g, and
40 = total solids fraction of the as-received compost aliquot, g.

17.1.4 Place the 1:5 slurry on a shaker for 20 min at 180 reciprocations or excursions per minute.

17.1.5 Maintain slurry at ambient laboratory temperature, (e.g., 20-23°C).

17.1.6 Extract the 1:5 Slurry liquid fraction.

17.1.6.1 Transfer the slurry to a 200-mL centrifuge tube. Centrifuge at 8000 *g* to separate solid and liquid fractions.

17.2 *Ion-Selective Electrode:*

17.2.1 Refer to the manufacturer's user guide for electrode calibration.

17.2.2 *Calibrate Electrometer or Specific Ion Meter*—Refer to manufacturer's instructions.

17.2.3 *Prepare Standard Curve*—Refer to manufacturer's instructions.

17.2.4 *Determine the Ammonia Present*

17.2.4.1 Dilute samples as needed to bring the concentration within the calibration curve range.

17.3 *Colorimetry:*

17.3.1 Determine the ammonium present in centrifugate.

NOTE 1C—If the extract cannot be analyzed within 4 h, place the sample in 4°C refrigerated storage for no more than five days. Acidify if storage exceeds five days.

18. Calculations and Corrections for Method C

18.1 Data and reporting units: NH_4^+–N mg kg^{-1}.

18.2 Calculate total concentration of ammonium in elemental form (NH_4^+–N) per kg of compost.

18.2.1 *Ion-Selective Electrode:*

$$A = B \times [C + (D - E)] \div E \qquad \textit{Equation 18.2.1}$$

ASSUME—1 mL ≡ 1 g; and 1 mg L^{-1} ≡ 1 mg kg^{-1}

where:

A = elemental N as NH_4^+ per kg of oven-dried compost, mg kg^{-1},
B = measured concentration of NH_4^+–N, mg L^{-1},
C = volume of extracting solution added to sample, mL,
D = mass of the sample aliquot at as-received moisture, g, and
E = Oven dry weight at 70±5°C, g.

18.2.2 *Colorimetry:*

$$A = B \times [C + (D - E)] \div [E \times 1.286] \qquad \textit{Equation 18.2.2}$$

ASSUME—1 mL ≡ 1 g; and 1 mg L^{-1} ≡ 1 mg kg^{-1}

where:

A = elemental N as NH_4^+ per kg of compost, mg kg^{-1}.
B = measured concentration of NH_4^+ (mg L^{-1}),
C = volume of extraction solution, mL, and
D = mass of the sample aliquot at as as-received moisture, g,
E = Oven dry weight at 70±5°C g,
1.286 = factor to convert NH_4^+ to elemental N (NO_3^-–N).

Test Method: Nitrogen. Total Nitrogen by Oxidation						**Units:** % dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
04.02-D	04.02-D				04.02-D			04.02-D

04.02-D TOTAL NITROGEN BY OXIDATION

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

19. Selection of Method

19.1 *Oxidation by Dry Combustion:*

19.1.1 *Dumas Method (Preferred)*—Automated apparatus options. Refer to manuals from manufactures of analyzers using the Dumas method.

19.2 *Sample Preparation and Pretreament*

19.2.1 *Heterogeneous and coarse compost low in volatile N*—Use air-dried material with inerts removed that is finely milled to a powder. Store samples in separate sealed containers at ambient laboratory temperature for no more than two or three weeks. Store material in a frozen state (-4°C) after three weeks to minimize enzymatic degradation of the sample.

19.2.2 *Homogenous, immature compost and feedstock high in volatile N*—Use as-received moist material. Gently rub the sample through a 4-mm sieve and thouroughly mix sieved sample material. Determine sample moisture on a parallel aliquot. Avoid cross contamination of volatile NH_4^+ among samples.

19.2.2.1 Ammonia loss through volatilization during the air-drying step of sample preparation may result in a significantly lower total nitrogen determination for some samples.

19.3 *Wet Oxidation Technique:*

19.3.1 *Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique:*

19.3.1.1 Include nitrate reduction steps outlined in Method 04.02-A, or

19.3.1.2 Add %N from TKN determination without nitrate reduction step and %N from NO_3–N determined using *Method 04.02-B Nitrate Nitrogen Determination.*

04.02 METHODS SUMMARY

20. Report

20.1 *Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique:*

20.1.1 *Reporting Units*—Total Kjeldahl Nitrogen (TKN, %) or Total Nitrogen (TN, %) where nitrate reduction is applied.

20.1.2 *Significant Figures*—±0.1%.

20.1.3 *Lower Reporting Limit*—0.1 %, using a routine dilution factor of 333.3×.

20.2 *Method 04.02-B Nitrate Nitrogen Determination*—Report methods selected for determinations nitrate nitrogen.

20.2.1 *Technique*—Report technique used.

20.2.2 *Reporting Units*—mg–N kg^{-1}-Compost on an oven dry weight basis (70±5°C).

20.2.3 *Significant Figures*—±0.1 mg kg^{-1}.

20.2.4 *Lower Reporting Limit*—2 mg kg^{-1} N in compost on an oven-dried basis (70±5°C).

20.3 *Method 04.02-C Ammonium Nitrogen Determination*—Report methods selected for determinations ammonium nitrogen.

20.3.1 *Technique*—Report technique used.

20.3.2 *Reporting Units*—mg–N kg^{-1}-Compost on an oven dry weight basis (70±5°C).

20.3.3 *Significant Figures*—±0.1 mg kg^{-1}.

20.3.4 *Lower Reporting Limit*—2 mg kg^{-1} N in compost on a dry weight basis (70±5°C).

20.4 *Method 04.02-D Total Nitrogen by Oxidation*—Report apparatus used and methods selected for determinations of total nitrogen.

20.4.1 *Technique*—Report technique used.

20.4.2 *Reporting Units*—% N.

20.4.3 *Significant Figures*—±0.01.

20.4.4 *Lower Reporting Limit*—0.01.

21. Precision and Bias

21.1 *Duplicate Samples*—acceptable variability across duplicates should be less than ±5%, depending on method. If variability exceeds ±5%, repeat duplication with another sample from the same batch. Lack of precision is generally due to inadequate sample mixing and blending prior to this determination:

$$V = |A - B| \times 100 \div C \qquad \textit{Equation 21.1}$$

where:

V = percent variability,

A = first duplicate,

B = second duplicate, and

C = average of usplicates

21.2 *Reference Sample*—Warning limit is the 95% confidence interval based upon periodically updated cumulative mean and standard deviation of the reference sample. Rejection limit is outside the 99% confidence interval (approximately three standard deviations). If standard reference result is rejected, repeat with the next batch of samples along with 1-2% of the unknown samples that were run with the batch containing the rejected sample.

21.3 *NIST Reference Checks*—Warning limit is within 95% confidence interval published by NIST. If outside of this limit, use confidence limits of this laboratories past performance.

21.4 *Method 04.02-A Total Kjeldahl Nitrogen, Semi-Micro Kjeldahl Technique*—The precision of this test was evaluated by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test was not determined. Data are being sought for use in developing a bias statement.

21.4.1 Precision was determined for nine U of MN standard reference MSW compost samples and 10 subsamples taken from a field composite sample for each of three sites at two separate sampling periods (1993). Variability is expressed as standard deviation (Std Dev) and coefficient of deviation (%CV).

Table 04.02-A1 Total Kjeldahl Nitrogen. Intra-sample precision from three sites and two sample dates, %TKN.

Median	*Std Dev*	*% CV*	*Number of Samples*
1.21	0.04	3.0	10
1.18	0.01	1.3	10
1.10	0.03	2.9	10
0.99	0.04	3.5	10
1.36	0.07	5.0	10
1.30	0.04	3.0	10

21.5 *Method 04.02-B Nitrate Nitrogen Determination by Colorimetry*—The precision of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test was not determined. Data are being sought for use in developing a bias statement.

21.5.1 Precision was determined for 10 subsamples taken from a field composite sample for each of three sites at two separate sampling periods (1993).

Variability is expressed as standard deviation (Std Dev) and coefficient of deviation (%CV).

Table 04.02-B1 Nitrate Nitrogen by Colorimetry precision, mg kg^{-1} dw basis.

Median	*Std Dev*	*% CV*	*Number of Samples*
2.6	1.0	37.7	10
2.0	0.0	0.0	10
16.0	0.6	3.4	10
2.0	0.0	0.0	10
2.0	0.0	0.0	10
2.0	0.0	0.0	10

21.6 *Method 04.02-C Ammonium Nitrogen Determination by Colorimetry*—The precision of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test was not determined. Data are being sought for use in developing a bias statement.

21.6.1 Precision was determined for 10 subsamples taken from a field composite sample for each of three sites at two separate sampling periods (1993). Variability is expressed as standard deviation (Std Dev) and coefficient of deviation (%CV).

Table 04.02-C1 Ammonium by colorimetry precision, mg kg^{-1} dw basis.

Median	*Std Dev*	*% CV*	*Number of Samples*
1425	45	3.1	10
1480	46	3.1	10
956	324	32.6	10
799	99	12.6	10
463	123	24.9	10
853	24	2.8	10

21.7 *Method 04.02-D Total Nitrogen by Oxidation*—Precision and bias of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN.

21.7.1 *Precision*—Determined for nine U of MN standard reference MSW compost samples and 10 subsamples taken from a field composite sample for each of three sites at two separate sampling periods (1993). Variability is expressed as standard deviation (Std Dev) and coefficient of deviation (%CV).

Table 04.02-D1 Total nitrogen. Intra-sample precision from three sites and two sample dates, TN% dw basis, Total Kjeldahl Nitrogen with Nitrate Reduction Step.

Median	*Std Dev*	*% CV*	*Number of Samples*
1.21	0.04	3.0	10
1.18	0.01	1.3	10
1.10	0.03	2.9	10
0.99	0.04	3.5	10
1.36	0.07	5.0	10
1.30	0.04	3.0	10

22. Keywords

22.1 ammonia; ammonium; denitrification; nitrate; nitrification; nitrogen; total nitrogen; total Kjeldahl nitrogen; TKN; organic nitrogen; nitrate reduction; combustion; oxidation

Test Method: Phosphorus. Two Methods							**Units:** See method	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.03-A							04.03-A
	04.03-B			04.03-B				04.03-B

04.03 PHOSPHORUS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers tests for determinations of phosphorus in compost and compost feedstocks.

1.1.1 *Method 04.03-A Total Phosphorus*

1.1.2 *Method 04.03-B Water-Soluble Phosphorus*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC Sections:*

Section 04.12 Digestion Techniques

Section 04.14 Inductively Coupled Plasma

2.2 *Other References:*

Functions of Mineral Nutrients: Macronutrients. pp. 195-268. *In* Mineral Nutrition of Higher Plants. Marschner, H., ed., Institute of Plant Nutrition. University of Hohenheim, Germany. Academic Press Limited. 1986. 24/28 Oval Road, London NW1 7DX.

The effects of mineral deficiencies and excesses on growth and composition. *In* Diagnosis of Mineral Disorders in Plants. Vol 1. C. Bould, E.J. Hewitt FRS, and P. Needham, eds., Chemical Publishing, NY. 1984

3. Terminology

3.1 *phosphorus, n*—(symbol P) A highly reactive, poisonous, nonmetallic element occurring naturally as phosphate (PO_4) in minerals, especially apatite, and existing in three allotropic forms, white (or sometimes yellow), red, and black. An essential constituent of protoplasm, it is used in safety matches, pyrotechnics, incendiary shells, fertilizers and as an anti-corrosion protectant for metal surfaces. Atomic number 15; atomic weight 30.9738; melting point (white) 44.1°C; boiling point 280°C; specific gravity (white) 1.82; valence 3, 5.

4. Summary of Test Methods

4.1 *Method 04.03-A Total Phosphorus*—An air-dried, milled sample is digested and the relative P content of the digestate is determined using ICP. Concentration is reported on a sample dw basis determined at 70±5ºC.

4.2 *Method 04.03-B Water-Soluble Phosphorus*—A 1:20 solids:liquid mixture of as-received compost and deionized water is blended at ambient laboratory temperature (approximately 23ºC). The mixture is shaken in a 500-mL closed container at 180 excursions per minute for 20 minutes. The mixture is centrifuged at 8000 *g* for 15 minutes. The supernatant is passed through a 0.45 µm filter and P content is determined using inductively coupled plasma (ICP) emission spectrospy. Concentration is reported on a dw basis with samples dried at 70±5ºC.

5. Significance and Use

5.1 Phosphorus is a macronutrient required for plant growth. It is taken up by plants as $H_2PO_4^{-1}$, HPO_4^{-2} or HPO^{-3}, depending upon the pH of the soil. Most of the total soil P is part of chemical compounds that have limited solubility. In neutral to alkaline soils, calcium phosphate is formed, whereas in acid soils, iron and aluminum phosphates are produced. Phosphorus is present in all living cells and is used by plants and animals to form nucleic acids (DNA and RNA).

5.2 There is no universally accepted method to determine plant-available P in compost. Nonetheless, agronomists, horticulturists and landscapers need P

content data to make decisions regarding supplemental fertilizer requirements.

5.3 This edition of TMECC presents two test methods for P determinations: the readily soluble forms of P that are loosly bound onto solids; and total P which encompases almost all forms of P in a compost.

Test Method: Phosphorus. Total Phosphorus							**Units:** % dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.03-A							04.03-A

04.03-A TOTAL PHOSPHORUS

6. Selection of Method

6.1 *Digestion:*

6.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

6.1.2 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

6.1.3 Method 04.12-E—Aqua Regia Procedure.

6.2 *Determination:*

6.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES).

7. Report

7.1 *Total Phosphorus*

7.1.1 *Convert Elemental P to P_2O_5:*

$$P_2O_5 = P \times 2.2914 \qquad \textit{Equation 7.1}$$

where:

P_2O_5 = standard format for fertilizer labeling, % dw,

P = elemental form of phosphorus, mg kg^{-1} or %, and

2.2914 = conversion factor.

7.1.2 *Total Macronutrient Content*—Report concentration of P_2O_5 in compost as a percentage, dry weight basis.

7.1.2.1 *As a Fertilizer Grade*—Report each of the three macronutrients as percentages and report in the following order: N - P_2O_5 - K_2O

7.1.2.2 *Determination Technique*—Report digest method and determination methods.

Test Method: Phosphorus. Water-Soluble Phosphorus							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.03-B			04.03-B				04.03-B

04.03-B WATER-SOLUBLE PHOSPHORUS

8. Selection of Method

8.1 *Digestion:*

8.1.1 Method 04.12-D—Water-Soluble Elements.

8.2 *Determination:*

8.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

9. Report

9.1 *Water-Soluble Phosphorus*

9.1.1 Report as mg of elemental P per kg of compost (mg kg^{-1}), dry weight basis.

9.1.2 *Determination Technique*—Report all deviations from the digest method as presented in Method 04.12-D and from determination Method 04.14-A.

Test Method: Potassium. Two Methods							**Units:** See methods	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.04-A							04.04-A
	04.04-B			04.04-B				04.04-B

04.04 POTASSIUM

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers tests for determinations of potassium in compost and compost feedstocks.

1.1.1 *Method 04.04-A Total Potassium*

1.1.2 *Method 04.04-B Water-Soluble Potassium*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC Sections:*

Section 04.12 Digestion Techniques

Section 04.13 Atomic Absorption Spectrometry

Section 04.14 Inductively Coupled Plasma

2.2 *Other References:*

Functions of Mineral Nutrients: Macronutrients. pp. 195-268. In Mineral Nutrition of Higher Plants. Marschner, H., ed., Institute of Plant Nutrition. University of Hohenheim, Germany. Academic Press Limited. 1986. 24/28 Oval Road, London NW1 7DX.

The effects of mineral deficiencies and excesses on growth and composition. *In* Diagnosis of Mineral Disorders in Plants. Vol 1. C. Bould, E.J. Hewitt FRS, and P. Needham, eds., Chemical Publishing, NY. 1984

3. Terminology

3.1 *potassium, n*—(symbol K) A soft, silver-white, highly or explosively reactive metallic element that occurs in nature only in compounds. It is obtained by electrolysis of its common hydroxide and found in or converted to a wide variety of salts. It used in the manufacture of fertilizers and soaps. Atomic number 19; atomic weight 39.102; melting point 63.65°C; boiling point 774°C; specific gravity 0.862; valence 1.

4. Summary of Test Methods

4.1 *Method 04.04-A Total Potassium*—An air-dried, milled sample is digested and the K content of the digestate is determined using ICP.

4.2 *Method 04.04-B Water-Soluble Potassium*—A 1:20 solids:liquid mixture of as-received compost and deionized water is blended at ambient laboratory temperature (approximately 23ºC). The mixture is processed by shaking in a 500-mL closed container on a mechanical shaker set at 180 excursions per minute for 20 minutes. The mixture is centrifuged at 8000 *g* for 15 minutes. The supernatant is pressed through a 0.45 µm filter and the K content is determined using ICP. Concentration is reported on a sample dw basis determined at 70±5ºC.

5. Significance and Use

5.1 Potassium is a macronutrient required for plant growth, and is a cofactor for several important enzyme activities.

5.2 Agronomists, horticulturists and landscapers need K content data to make decisions regarding supplemental fertilizer requirements. Unfortunately, there is no universal method to determine plant-available K in compost. This edition of TMECC presents two methods to measure K; the readily soluble forms of K that are loosely bound onto organic solids; and total K which comprises all forms of K in a compost.

Test Method: Potassium: Total Potassium							**Units:** % dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.04-A							04.04-A

04.04-A TOTAL POTASSIUM

6. Selection of Method

6.1 *Digestion:*

6.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

6.1.2 Method 04.12-B—Nitric Acid Digestion, US EPA 3050B Modified.

6.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

6.1.4 Method 04.12-E—Aqua Regia Procedure.

6.2 *Determination:*

6.2.1 Method 04.13—Atomic Absorption Spectrometry.

6.2.2 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

7. Report

7.1 *Total Potassium*

7.1.1 Convert Elemental K to K_2O:

$K_2O = K \times 1.2046$ *Equation 7.1.1*

7.1.2 *Total Macronutrient Content*—Report concentration of K_2O in compost as a percentage, dry weight basis.

7.1.2.1 *As a Fertilizer Grade*—Report each of the three macronutrients as percentages and report in the following order: N - P_2O_5 - K_2O.

7.1.2.2 *Determination Technique*—Report digest method and determination technique.

Test Method: Potassium: Water-Soluble Potassium							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.04-B			04.04-B				04.04-B

04.04-B WATER-SOLUBLE POTASSIUM

8. Selection of Method

8.1 *Digestion:*

8.1.1 Method 04.12-D—Water-Soluble Elements.

8.2 *Determination:*

8.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

8.2.2 US EPA Method 7610—Potassium Atomic Absorption Spectrophotometry, Direct Aspiration.

9. Report

9.1 *Water-Soluble Potassium*

9.1.1 Report as mg of elemental K per kg of compost (mg kg^{-1}), dry weight basis.

9.1.2 *Determination Technique*—Report all deviations from the digest method as presented in Method 04.12-D and from determination Method 04.14-A.

Test Method: Secondary and Micronutrient Content. Twelve Elements							**Units:** element specific	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.05-Mg
								04.05-Ca
								04.05-S
	04.05-Na							04.05-Na
								04.05-B
	04.05-Cl							04.05-Cl
								04.05-Co
04.06-Cu	04.06-Cu						04.06-Cu	04.05-Cu
								04.05-Fe
								04.05-Mn
04.06-Mo	04.06-Mo						04.06-Mo	04.05-Mo
04.06-Zn	04.06-Zn						04.06-Zn	04.05-Zn

04.05 SECONDARY AND MICRONUTRIENT CONTENT

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the determination of elements that are considered secondary- and micronutrients for plant growth.

1.1.1 *Method 04.05-Mg Magnesium.*

1.1.2 *Method 04.05-Ca Calcium.*

1.1.3 *Method 04.05-S Sulfur.*

1.1.4 *Method 04.05-Na Sodium.*

1.1.5 *Method 04.05-B Boron.*

1.1.6 *Method 04.05-Cl Chloride.*

1.1.7 *Method 04.05-Co Cobalt.*

1.1.8 *Method 04.05-Cu Copper.*

1.1.9 *Method 04.05-Fe Iron.*

1.1.10 *Method 04.05-Mn Manganese.*

1.1.11 *Method 04.05-Mo Molybdenum.*

1.1.12 *Method 04.05-Zn Zinc.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC Sections:*

Section 04.12 Digestion Techniques

Section 04.13 Atomic Absorption Spectrometry

Section 04.14 Inductively Coupled Plasma

2.2 *Other References:*

Marschner, H. 1986. Nitrogen Fixation. pp. 173-194. In Mineral Nutrition of Higher Plants. Institute of Plant Nutrition. University of Hohenheim, Germany. Academic Press Limited. 24/28 Oval Road, London NW1 7DX.

Marschner, H. 1986. Functions of Mineral Nutrients: Macronutrients. pp. 195-340. *In* Mineral Nutrition of Higher Plants. Institute of Plant Nutrition. University of Hohenheim, Germany. Academic Press Limited. 24/28 Oval Road, London NW1 7DX.

Marschner, H. 1986. Beneficial Mineral Elements. pp. 340-368. *In* Mineral Nutrition of Higher Plants. Institute of Plant Nutrition. University of Hohenheim, Germany. Academic Press Limited. 24/28 Oval Road, London NW1 7DX. 1986.

The effects of mineral deficiencies and excesses on growth and composition. *In* Diagnosis of Mineral Disorders in Plants. Vol 1. C. Bould, E.J. Hewitt FRS, and P. Needham, eds., Chemical Publishing, NY. 1984

Trace elements in soils and plants. Alina Kabata-Pendias, Henryk Pendias, eds. 2nd edition. CRC Press, Inc., 2000 Corporate Blvd., NW, Boca Raton, FL 33431. 1992.

3. Terminology

3.1 *boron, n*—(symbol B) A soft, brown, amorphous or crystalline nonmetallic element, extracted chiefly from kernite and borax and used in flares, propellant mixtures, nuclear reactor control elements, abrasives, and hard metallic alloys. Atomic number 5; atomic weight 10.811; melting point 2,300°C; sublimation point 2,550°C; specific gravity (crystal) 2.34; valence 3.

3.2 *calcium, n*—(symbol Ca) A silvery, moderately hard metallic element that constitutes approximately 3% of the earth's crust and is a basic component of most animals and plants. It occurs naturally in limestone, gypsum, and fluorite, and its compounds are used to make plaster, quicklime, Portland cement, and metallurgic and electronic materials. Atomic number 20; atomic weight 40.08; melting point 842 to 848°C; boiling point 1,487°C; specific gravity 1.55; valence 2.

3.3 *chloride, n*——(symbol Cl) A chlorine-containing compound formed by the reaction of chlorine (Cl_2) with one of many different elements, both metals and nonmetals. Chlorides formed through reactions with chlorine have high oxidation numbers, (e.g., iron(III) chloride ($FeCl_3$), tin(IV) chloride ($SnCl_4$), or antimony(V) chloride ($SbCl_5$)). Chlorine is relatively inert toward carbon, nitrogen, and oxygen.

3.4 *chlorine, n*—(symbol Cl element gas Cl_2) Chlorine occurs in nature primarily as sodium chloride NaCl in seawater and is capable of combining with nearly all other elements. Chlorine (Cl_2) is a greenish-yellow diatomic halogen gas that is produced principally by electrolysis of sodium chloride (brine) and is used widely to purify water, as a disinfectant and bleaching agent. Atomic number 17; atomic weight 35.45; freezing point -100.98°C; boiling point -34.6°C; specific gravity 1.56 (-33.6°C); valence 1, 3, 5, 7.

3.5 *cobalt, n*—(symbol Co) A hard, brittle metallic element, found associated with nickel, silver, lead, copper, and iron ores and resembling nickel and iron in appearance. It is used chiefly for magnetic alloys, high-temperature alloys, and in the form of its salts for blue glass and ceramic pigments. Atomic number 27; atomic weight 58.9332; melting point 1,495°C; boiling point 2,900°C; specific gravity 8.9; valence 2, 3.

3.6 *copper, n*—(symbol Cu) A ductile, malleable, reddish-brown metallic element that is an excellent conductor of heat and electricity and is widely used for electrical wiring, water piping, and corrosion-resistant parts, either pure or in alloys such as brass and bronze. Atomic number 29; atomic weight 63.54; melting point 1,083°C; boiling point 2,595°C; specific gravity 8.96; valence 1, 2.

3.7 *iron, n*—(symbol Fe) A silvery-white, lustrous, malleable, ductile, magnetic metallic element occurring abundantly in combined forms, notably in hematite, limonite, magnetite, and taconite, and used alloyed in a wide range of important structural materials. Atomic number 26; atomic weight 55.847; melting point 1,535°C; boiling point 2,750°C; specific gravity 7.874 (at 20°C); valence 2, 3, 4, 6.

3.8 *magnesium, n*—(symbol Mg) A light, silvery-white, moderately hard metallic element that in ribbon or powder form burns with a brilliant white flame. It is used in structural alloys, pyrotechnics, flash photography, and incendiary bombs. Atomic number 12; atomic weight 24.312; melting point 649°C; boiling point 1,090°C; specific gravity 1.74 (at 20°C); valence 2.

3.9 *manganese, n*—(symbol Mn) A gray-white or silvery brittle metallic element, occurring in several allotropic forms, found worldwide, especially in the ores pyrolusite and rhodochrosite and in nodules on the ocean floor. It is alloyed with steel to increase strength, hardness, wear resistance, and other properties and with other metals to form highly ferromagnetic materials. Atomic number 25; atomic weight 54.9380; melting point 1,244°C; boiling point 1,962°C; specific gravity 7.21 to 7.44; valence 1, 2, 3, 4, 6, 7.

3.10 *molybdenum, n*—(symbol Mo) A hard, silvery-white metallic element used to toughen alloy steels and soften tungsten alloy. An essential trace element in plant nutrition, it is used in fertilizers, dyes, enamels, and reagents. Atomic number 42; atomic weight 95.94; melting point 2,617°C; boiling point 4,612°C; specific gravity 10.22 (at 20°C); valence 2, 3, 4, 5.

3.11 *sodium, n*—(symbol Na) A soft, light, extremely malleable silver-white metallic element that reacts explosively with water, is naturally abundant in combined forms, especially in common salt, and is used in the production of a wide variety of industrially important compounds. Atomic number 11; atomic weight 22.99; melting point 97.8°C; boiling point 892°C; specific gravity 0.971; valence 1.

3.12 *sulfur, n*—(symbol S) A pale yellow nonmetallic element occurring widely in nature in several free and combined allotropic forms. It is used in black gunpowder, rubber vulcanization, the manufacture of insecticides and pharmaceuticals, and in the preparation of sulfur compounds such as hydrogen sulfide and sulfuric acid. Atomic number 16; atomic weight 32.064; melting point (rhombic) 112.8°C; (monoclinic) 119.0°C; boiling point 444.6°C; specific gravity (rhombic) 2.07; (monoclinic) 1.957; valence 2, 4, 6.

3.13 *zinc, n*—(symbol Zn) A bluish-white, lustrous metallic element that is brittle at room temperature but malleable with heating. It is used to form a wide variety of alloys including brass, bronze, various solders, and nickel silver, in galvanizing iron and other metals, for electric fuses, anodes, and meter cases, and in roofing,

gutters, and various household objects. Atomic number 30; atomic weight 65.37; melting point 419.4°C; boiling point 907°C; specific gravity 7.133 (25°C); valence 2.

4. Summary of Test Methods

4.1 Refer to specific elements.

5. Significance and Use

5.1 Compost provides essential nutrients for plant growth. Essential nutrients are classified as macronutrients that are required in large quantities and micronutrients that are required in small to trace quantities. Macronutrients are further subdivided as primary and secondary nutrients; N, P and K are the three primary nutrients and S, Mg and Ca are the secondary nutrients. Micronutrients include Fe, Mn, Zn, Cu, B and Mo. Na is sometimes utilized by plants, but is not considered essential. Co is an essential element for N_2 fixing symbiotic bacteria and included because of its indirect importance in plant growth and crop production.

5.2 Essential elements for plant growth include carbon, hydrogen, oxygen, nitrogen, phosphorous, potassium, calcium, magnesium, sulfur, iron, boron, chlorine, copper, manganese, molybdenum, and zinc.

5.3 *Macronutrient Status*—Including nitrogen, carbon (not a macronutrient but an essential element for plant growth), phosphorous, and potassium, are measured as the percent of dry solids. Nitrogen and carbon affect the microbial metabolism by altering the carbon to nitrogen ratio. Carbon affects mineralization rates when applied to the soil. Nitrogen, phosphorous, and potassium determine the value of the end product as an organic fertilizer.

5.4 *Micronutrient Status*—Sometimes called trace elements, they include boron, chloride, cobalt, copper, iron, manganese, molybdenum, and zinc. Micronutrients are required by plants for growth in small quantities, but can be toxic at high levels. Stimulators of the enzymatic function necessary to keep the composting process going include the micronutrients, and can include macronutrients and nickel and sodium. Copper, molybdenum, and zinc are heavy metals for which life-time and annual loading rates on cropland have been set, along with arsenic, cadmium, chromium, lead, mercury, nickel, and selenium.

5.5 *Agriculture Index (AgIndex)*—Measure of nutrient content ($TN + P_2O_5 + K_2O$) divided by salt ($Cl + Na$) content in compost or soils. An AgIndex value < 2 is rated poor and an AgIndex > 10 is rated excellent for seed germination and growth. Refer to Method 05.01-F for more information on Ag Index interpretation.

Test Method: Secondary and Micro-Nutrient Content. Magnesium						**Units:** % dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.05-Mg

04.05-MG MAGNESIUM

6. Significance

6.1 Magnesium is a macronutrient. Plants take up magnesium ions (Mg^{+2}). Chlorophyll contains magnesium; both are essential for photosynthesis. Magnesium serves as a cofactor for many plant enzymes required for growth. Magnesium is mobile within plants and is readily translocated from older to younger tissue under conditions of deficiency. Magnesium carbonate and magnesium oxides can increase compost pH, and reduce phytotoxicity of boron and other trace elements including molybdenum, nickel, and lead. Gypsum ($MgSO_4$) is sometimes used as a nutrient supplement because it has little or no impact on compost or soil pH status.

7. Selection of Method

7.1 *Digestion:*

7.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

7.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

7.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

7.1.4 Method 04.12-D—Water-Soluble Elements.

7.1.5 Method 04.12-E—Aqua Regia Procedure.

7.2 *Determination:*

7.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

7.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Secondary and Micro-Nutrient Content. Calcium						**Units:** % dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.05-Ca

04.05-CA CALCIUM

8. Scope and Significance

8.1 Calcium is a macronutrient and is taken up by plants as the calcium ion (Ca^{+2}). It is an essential part of cell wall structure and must be present for the formation of new cells. Calcium carbonate and calcium oxides increase compost pH and reduce phytotoxicity of boron and other trace elements including molybdenum, nickel, and lead.

9. Selection of Method

9.1 *Digestion:*

9.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

9.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

9.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

9.1.4 Method 04.12-D—Water-Soluble Elements.

9.1.5 Method 04.12-E—Aqua Regia Procedure.

9.2 *Determination:*

9.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

9.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Secondary and Micro-Nutrient Content. Sulfur						**Units:** % dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.05-S

04.05-S SULFUR

10. Significance

10.1 Sulfur is a secondary plant nutrient. Excess sulfur may cause compost to become acidic and increase availability of some elements, including nickel, chromium, cadmium, lead, copper, and zinc. During anaerobic conditions, sulfur can produce odorous hydrogen sulfide (H_2S). In cases of sulfur deficiency, gypsum ($MgSO_4$) is often used as an inexpensive nutrient supplement; it has little or no impact on compost or soil pH status.

11. Selection of Method

11.1 *Digestion:*

11.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

11.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

11.1.3 Method 04.12-D—Water-Soluble Elements.

11.2 *Determination:*

11.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

11.2.2 Sulfur Analyzer (dry combustion)—Analytical Instruments For Plant Analysis. by M. E. Watson and R. A Isaac, Chapter 26 *In Soil Testing and Plant Analysis*, Third Edition, published by: Soil Science Society of America, Madison, Wisconsin.

11.2.3 Sulfate (SO_4) by Ion Chromatography—refer to manufacturer's instructions.

Test Method: Secondary and Micro-Nutrient Content. Sodium						**Units:** % dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.05-Na							04.05-Na

04.05-NA SODIUM

12. Significance

12.1 Sodium is an element that is generally not required for plant growth. Sodium in association with certain corresponding anions increases electrical conductivity in compost and soils.

NOTE NA1—Sodium from human perspiration may contaminate a compost sample, inflating the concentration. Care should be taken to prevent this kind of contamination of samples.

12.2 Sodium is one of the five elements used in calculating the AgIndex of composting feedstock blends, Method 05.02-F.

13. Selection of Method

13.1 *Digestion:*

13.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

13.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

13.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

13.1.4 Method 04.12-D—Water-Soluble Elements.

13.1.5 Method 04.12-E—Aqua Regia Procedure.

13.2 *Determination:*

13.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

13.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Secondary and Micro-Nutrient Content. Boron						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.05-B

04.05-B BORON

14. Significance

14.1 Boron is a micronutrient that is essential in small quantities for plant growth. It is taken up by plants as the borate ion (BO_3^{-3}). At higher concentrations boron can induce toxicity to plants especially under acidic conditions. Handbooks are available that list relative tolerance of plants to boron. See "Soil Tolerance of Plants," E. V. Mass, in *Handbook of Plant Science*.

14.2 Boron is bound into various small and large molecules and is combined with proteins. It is involved in the metabolism and transport of carbohydrates, flavenoid synthesis, nucleic acid synthesis, phosphate utilization, and polyphenol production. It is a constituent of phosphogluconates.

14.3 Deficient concentrations in agronomic crop plant leaf tissues range from 5-30 mg kg^{-1}. Sufficient concentrations in agronomic crop plant leaf tissues generally range from 10-100 mg kg^{-1}. Excessive and toxic concentrations range from 50-200 mg kg^{-1}. The common deficiency symptom is chlorosis of leaf margins. This is caused by excessive accumulation of nitrate that destroys embryonic tissues.

15. Selection of Method

15.1 *Digestion:*

15.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

15.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

15.1.3 Method 04.12-D—Water-Soluble Elements.

15.2 *Determination:*

15.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

Test Method: Secondary and Micro-Nutrient Content. Chloride						**Units:** % dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.05-Cl							04.05-Cl

04.05-CL CHLORIDE

16. Significance

16.1 Chloride (Cl^-) is a micronutrient that is essential in small quantities for plant growth. In high concentrations it is toxic to some plants. All common chlorides are soluble and contribute to the total salt content (salinity) of soils. Tables show tolerance of some plants to chloride levels in saturated extract of soil. Beets and barley show the highest tolerance near 90 cmol kg^{-1}, and avocado and strawberry show the least tolerance at 5 cmol kg^{-1}.

16.2 Chloride is one of the five elements used in calculating the AgIndex of composting feedstock blends, Method 05.02-F.

17. Selection of Method

17.1 *Digestion:*

17.1.1 Method 04.12-D Water-Soluble Elements

17.2 *Determination:*

17.2.1 Ion Chromatography, refer to manufacturer's instructions.

17.2.2 Ion-Selective Electrode, refer to manufacturer's instructions.

Test Method: Secondary and Micro-Nutrient Content. Cobalt						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.05-Co

04.05-CO COBALT

18. Significance

18.1 Cobalt is an essential trace element needed by symbiotic N_2 fixing bacteria. In plants, it is bound into miscellaneous small and large molecules and combined with proteins, including enzymes. It is involved in symbiotic N_2 fixation. It is a constituent of the coenzyme cobamide.

18.2 Sufficient concentrations in agronomic crop plant leaf tissues range form 0.02 to 1.0 mg kg^{-1}, up to a tolerable concentration 5.0 mg kg^{-1}. Excessive and toxic concentrations range from 15-30 mg kg^{-1}.

19. Selection of Method

19.1 *Digestion:*

19.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

19.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

19.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

19.1.4 Method 04.12-D—Water-Soluble Elements.

19.1.5 Method 04.12-E—Aqua Regia Procedure.

19.2 *Determination:*

19.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

19.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Secondary and Micro-Nutrient Content. Copper						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Cu	04.06-Cu						04.06-Cu	04.05-Cu

04.05-CU COPPER

20. Significance

20.1 Copper is a micronutrient essential in small quantities for plant growth. Copper is a cofactor of several enzymes in plants. It may play a role in Vitamin A production. A deficiency of copper inhibits protein synthesis. Plants take up copper as Cu^{+1} and Cu^{+2}. Some soils, usually organic soils, may be deficient in copper. In plants, it is bound into various small and large molecules and combines with proteins, including enzymes and is found in organelles such as mitochondria. It is involved in oxidation, photosynthesis, protein, carbohydrate and cell wall metabolism, possibly N_2 fixation. It is a constituent of various oxidases, plastocyanins, and ceniloplasmins.

20.2 Deficient concentrations in agronomic crop plant leaf tissues range from 2-5 mg kg^{-1}. Sufficient concentrations in agronomic crop plant leaf tissues range form 5-30 mg kg^{-1}, up to a commonly tolerable concentration of 50 mg kg^{-1}. Excessive and toxic concentrations range from 20-100 mg kg^{-1}.

20.3 Plant deficiency symptoms are usually expressed as wilting, melanism, white twisted tips and reduction of panicle formation in small grains, usually oats. Deficiencies in copper may inhibit lignification and the production or development of pollen.

20.4 Copper is a transition metal considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost. Copper in compost or soil poses no human health risk. Copper is readily taken up and translocated by plants. However, plant health is severely compromised at concentrations below those toxic to humans. Copper can potentially cause phytotoxicity, and can be an animal health concern through direct ingestion. The copper levels in mixed solid waste composts are typically below 200 mg kg^{-1} and pose minimal health risk to plants.

21. Selection of Method

21.1 *Digestion:*

21.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

21.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

21.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

21.1.4 Method 04.12-D—Water-Soluble Elements.

21.1.5 Method 04.12-E—Aqua Regia Procedure.

21.2 *Determination:*

21.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

21.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Secondary and Micro-Nutrient Content. Iron						**Units:** % dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.05-Fe

04.05-FE IRON

22. Significance

22.1 Iron is a secondary nutrient required for plant growth, and is a cofactor for several important enzyme activities. It is bound into miscellaneous small and large molecules combined with proteins, including enzymes. It is involved in photosynthesis, symbiotic N_2 fixation and redox reactions. It is a cofactor for biochemical processes such as respiration, photosynthesis and symbiotic nitrogen fixation. It is a constituent of hemo-proteins and nonheme iron proteins, dehydrogenases, and ferrodoxins. Iron is required for the formation of chlorophyll in plant cells. Plant deficiency symptoms are usually expressed as interveinal chlorosis of young organs.

22.2 Iron is taken up by plants either as ferrous ($Fe^{+2)}$ or ferric (Fe^{+3}) ions. Iron is a transition metal and high concentrations in soil may reduce detrimental effects of lead and cadmium.

23. Selection of Method

23.1 *Digestion:*

23.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

23.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

23.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

23.1.4 Method 04.12-D—Water-Soluble Elements.

23.1.5 Method 04.12-E—Aqua Regia Procedure.

23.2 *Determination:*

23.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

23.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Secondary and Micro-Nutrient Content. Manganese						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.05-Mn

04.05-MN MANGANESE

24. Significance

24.1 Manganese is a micronutrient required in small quantities for plant growth. Manganese serves as a cofactor for enzymes in growth processes. It, like iron, is involved in chlorophyll formation. High manganese concentrations may reduce iron deficiency. Manganese uptake in plants is primarily as the divalent ion (Mn^{+2}).

25. Selection of Method

25.1 *Digestion:*

25.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

25.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

25.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

25.1.4 Method 04.12-D—Water-Soluble Elements.

25.1.5 Method 04.12-E—Aqua Regia Procedure.

25.2 *Determination:*

25.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

25.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Secondary and Micro-Nutrient Content. Molybdenum							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Mo	04.06-Mo						04.06-Mo	04.05-Mo

04.05-MO MOLYBDENUM

26. Significance

26.1 Molybdenum is an essential trace element for plant growth. It is bound into miscellaneous large molecules and combined with proteins, including enzymes, and found in organelles such as mitochondria. It is involved in symbiotic N_2 fixation, nitrate reduction and valence charges. It is a constituent of nitrate reductase, nitrogenase, oxidases and molybdoferredoxin.

26.2 Molybdenum is taken up by plants as the molybdate ion, MoO_4^{-2}. It is required by plants to utilize nitrogen. Plants cannot transform nitrogen into amino acids without molybdenum. Legumes cannot fix atmospheric nitrogen symbiotically unless molybdenum is present. Deficiencies of molybdenum have required small additions to legumes grown in some regions.

26.3 Molybdenum is found in some regions of the country in quantities toxic to livestock.

26.4 Deficient concentrations in agronomic crop plant leaf tissues range from 0.1-0.3 mg kg^{-1}. Sufficient concentrations in agronomic crop plant leaf tissues range form 0.02 to 5.0 mg kg^{-1}, up to a tolerable concentration less than 10.0 mg kg^{-1}. Excessive and toxic concentrations range from 10-50 mg kg^{-1}. The common deficiency symptom is chlorosis of leaf margins and is due to excessive accumulation of nitrate, which then destroys embryonic tissue.

26.5 Molybdenum is considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost. Molybdenum can be a human health concern through direct ingestion. It can also be a health concern to animals through direct ingestion or through ingestion of forage with high molybdenum concentrations. This is especially the case for forage grown in inland desert areas.

27. Selection of Method

27.1 *Digestion:*

27.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

27.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

27.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

27.1.4 Method 04.12-D—Water-Soluble Elements.

27.1.5 Method 04.12-E—Aqua Regia Procedure.

27.2 *Determination:*

27.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

27.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Secondary and Micro-Nutrient Content. Zinc							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Zn	04.06-Zn						04.06-Zn	04.05-Zn

04.05-ZN ZINC

28. Significance

28.1 Zinc is an essential micronutrient for plant growth. It is an essential constituent of several important enzyme systems in plants. It controls the synthesis of indoleactetic acid, an important plant growth regulator. It is bound into miscellaneous large molecules combined with proteins, including enzymes, and found in organelles such as mitochondria. It is involved in carbohydrate, nucleic acid and lipid metabolism. It is a constituent of anhydrases, dehydrogenases, proteinases, and peptides.

28.2 Deficient concentrations in agronomic crop plant leaf tissues range from 10-20 mg kg^{-1}. Sufficient concentrations in agronomic crop plant leaf tissues range form 27-150 mg kg^{-1}, up to a commonly tolerable concentration less than 300 mg kg^{-1}. Excessive and toxic concentrations range from 100-400 mg kg^{-1}.

28.3 Terminal growth areas are affected first when zinc is deficient. Zinc is taken up by plants as the divalent ion (Zn^{+2}). The common deficiency symptom is interveinal chlorosis in monocots, stunted growth and violet-red points on leaves. Zinc is the micronutrient most often needed by crops in some regions of the USA.

28.4 Zinc is a trace metal considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost.

29. Selection of Method

29.1 *Digestion:*

29.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

29.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

29.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

29.1.4 Method 04.12-D—Water-Soluble Elements.

29.1.5 Method 04.12-E—Aqua Regia Procedure.

29.2 *Determination:*

29.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

29.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

04.05 METHODS SUMMARY

30. Report

30.1 Correct element concentrations to an oven dry weight basis (forced-air oven dried at 70±5°C). Report the digest method and determination technique used for the test parameter, source material (e.g., MSW, yard waste, biosolids, etc.), and composting process step (e.g., feedstock preparation, compost curing, etc.).

30.2 *Units*—Report results as % dw, or as mg kg^{-1} dw.

- N—Refer to specific test for reporting units.
- P, K, Mg, Ca, S, Cl, Na, Fe—Report as % dw basis determined at 70±5°C.
- B, Co, Cu, Mn, Mo, Zn—Report as mg kg-1 dw basis determined at 70±5°C.

31. Precision and Bias

31.1 *Method 04.05-S Sulfur*—Precision and bias for this test are not determined. Data are being sought to construct a precision and bias statement.

31.2 *Method 04.05-Cl Chloride*—Precision and bias for this test are not determined. Data are being sought to construct a precision and bias statement.

31.3 *Method 04.05-Co Cobalt*—Precision and bias for this test are not determined. Data are being sought to construct a precision and bias statement.

31.4 Precision and bias for the following this tests were determined using ICP-AES by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN.

31.4.1 *Precision*—Determined for nine U of MN standard reference MSW compost samples and 10 subsamples taken from a field composite sample for each of three sites at two separate sampling periods (1993). Variability is expressed as standard deviation (Std Dev) and coefficient of deviation (%CV).

31.5 *Method 04.05-Mg Magnesium, % Mg:*

Table 04.05-Mg Magnesium. Intra-sample precision from three sites and two sample dates.

Median	*Std Dev*	*% CV*	*Number of Samples*
0.39	0.01	2.7	10
0.34	0.01	3.4	10
0.56	0.02	3.6	10
0.55	0.03	4.5	10
0.31	0.01	2.8	10
0.35	0.02	4.8	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

31.6 *Method 04.05-Ca Calcium, % Ca:*

Table 04.05-Ca Calcium. Intra-sample precision from three sites and two sample dates.

Median	*Std Dev*	*% CV*	*Number of Samples*
2.99	0.07	2.3	10
3.28	0.07	2.1	10
4.01	0.11	2.7	10
3.43	0.12	3.6	10
3.07	0.05	1.5	10
3.22	0.11	3.4	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

31.7 *Method 04.05-Na Sodium, % Na:*

Table 04.05-Na Sodium. Intra-sample precision from three sites and two sample dates.

Median	*Std Dev*	*% CV*	*Number of Samples*
0.60	0.01	1.7	10
0.64	0.02	2.4	10
0.73	0.02	2.0	10
0.70	0.02	3.3	10
0.75	0.01	1.8	10
0.80	0.03	3.3	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

31.8 *Method 04.05-B Boron, mg kg^{-1} B:*

Table 04.05-B Boron. Intra-sample precision from three sites and two sample dates.

Median	*Std Dev*	*% CV*	*Number of Samples*
73	4.3	5.8	10
61	1.6	2.6	10
103	3.5	3.3	10
144	4.3	3.0	10
114	3.3	2.9	10
124	4.5	3.7	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

31.9 *Method 04.05-Cu Copper, mg kg^{-1} Cu:*

Table 04.05-Cu Copper. Intra-sample precision from three sites and two sample dates.

Median	*Std Dev*	*% CV*	*Number of Samples*
441	34.2	7.7	10
342	13.7	3.9	10
754	172.0	20.8	10
788	103.4	12.9	10
296	65.5	20.4	10
316	15.0	4.7	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

31.10 *Method 04.05-Fe Iron, % Fe:*

Table 04.05-Fe Iron. Intra-sample precision from three sites and two sample dates.

Median	*Std Dev*	*% CV*	*Number of Samples*
1.95	0.07	3.8	10
1.64	0.06	3.5	10
2.71	0.09	3.2	10
2.72	0.12	4.3	10
1.65	0.06	3.8	10
1.83	0.10	5.6	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

31.11 *Method 04.05-Mn Manganese, mg kg^{-1} Mn:*

Table 04.05-Mn Manganese. Intra-sample precision from three sites and two sample dates.

Median	*Std Dev*	*% CV*	*Number of Samples*
385	14	3.6	10
337	12	3.4	10
1020	49	4.8	10
1010	45	4.4	10
834	39	4.6	10
886	45	5.0	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

31.12 *Method 04.05-Mo Molybdenum, mg kg^{-1} Mo:*

Table 04.05-Mo Molybdenum. Intra-sample precision from three sites and two sample dates.

Median	*Std Dev*	*% CV*	*Number of Samples*
6.16	0.26	4.2	10
6.41	0.81	12.0	10
7.85	0.20	2.5	10
6.40	0.41	6.4	10
5.55	1.00	17.1	10
5.47	0.39	7.1	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

31.13 *Method 04.05-Zn Zinc, mg kg^{-1} Zn:*

Table 04.05-Zn Zinc. Intra-sample precision from three sites and two sample dates.

Median	*Std Dev*	*% CV*	*Number of Samples*
909	32	3.5	10
785	19	2.5	10
1742	347	18.9	10
1784	254	13.5	10
1299	114	8.5	10
1436	102	7.0	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

32. Keywords

32.1 plant nutrients; nitrogen; phosphorus; potassium; calcium; magnesium; sodium; boron; cobalt; copper; sulfur; iron; manganese; molybdenum; zinc

Test Method: Heavy Metals and Hazardous Elements. Thriteen Elements							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-As	04.06-As						04.06-As	
04.06-Be	04.06-Be						04.06-Be	
04.06-Cd	04.06-Cd						04.06-Cd	
04.06-Cu	04.06-Cu						04.06-Cu	04.05-Cu
04.06-Cr	04.06-Cr						04.06-Cr	
04.06-Pb	04.06-Pb						04.06-Pb	
04.06-Hg	04.06-Hg						04.06-Hg	
04.06-Mo	04.06-Mo						04.06-Mo	04.05-Mo
04.06-Ni	04.06-Ni						04.06-Ni	
04.06-Se	04.06-Se						04.06-Se	
04.06-Sr	04.06-Sr						04.06-Sr	
04.06-V	04.06-V						04.06-V	
04.06-Zn	04.06-Zn						04.06-Zn	04.05-Zn

04.06 HEAVY METALS AND HAZARDOUS ELEMENTS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Source

1.1 This section covers methods of digestion and determination for heavy metals and hazardous elements in compost.

1.1.1 *Method 04.06-As Arsenic*.

1.1.2 *Method 04.07-Be Beryllium*.

1.1.3 *Method 04.06-Cd Cadmium*.

1.1.4 *Method 04.06-Cu Copper*.

1.1.5 *Method 04.06-Cr Chromium*.

1.1.6 *Method 04.06-Pb Lead*.

1.1.7 *Method 04.06-Hg Mercury*.

1.1.8 *Method 04.06-Mo Molybdenum*.

1.1.9 *Method 04.06-Ni Nickel*.

1.1.10 *Method 04.06-Se Selenium*.

1.1.11 *Method 04.06-Sr Strontium*.

1.1.12 *Method 04.06-V Vanadium*.

1.1.13 *Method 04.06-Zn Zinc*.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

1.3 *Issues*—Heavy Metals and Hazardous Elements including arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), and zinc (Zn) are potential environmental pollutants at certain concentrations and as such are of regulatory concern relative to compost feedstocks and finished compost uses. For example, cadmium is one of the most hazardous of the heavy metals. It is toxic to animals and humans at levels not toxic to plants because it inhibits calcium uptake in bones. Hg inhibits respiration at concentrations greater than 100 mg kg^{-1} dw. Generally, the most toxic metals for both higher plants and certain microorganisms are Hg, Cu, Ni, Pb, Co and Cd. Heavy Metals and Hazardous Elements including Cd, Cu, Pb, Hg, Ni, and Zn are elements for which lifetime and annual loading rates on cropland have been set, along with As, Cr, Mo, and Se.

1.3.1 The US EPA regulates land application of waste materials and substances containing heavy metals. Table 04.06-A1 is an example of the maximum application thresholds for biosolids. The concentration of each metal in the material is not to exceed the concentrations listed in *table 1* (Table 04.06-A1). The average monthly concentration corresponds to the maximum allowable concentration of a material. Repeated application of material containing heavy

metals should not cause concentrations in the soil to exceed the limits listed in *table 4* (Table 04.06-A1).

Table 04.06-A1 US EPA 40 CFR Part 503 in biosolids and biosolids compost limits.

Metals	*Table 1. Ceiling [mg kg^{-1}]*	*Table 3. Monthly average [mg kg^{-1}]*	*Table 4. Annual loading rate [kg ha^{-1} yr^{-1}]*
Arsenic	75	41	2.0
Cadmium	85	39	1.9
Copper	4300	1500	75
Lead	840	300	15
Mercury	57	17	0.85
Molybdenum	75		
Nickel	420	420	21
Selenium	100	100	5
Zinc	7500	2800	140

NOTE—Mass units reported on dw basis (70±5°C).

ADAPTED FROM—Table 3 of §503.13. Pollutant Concentrations, Table 4 of §503.13. Annual Pollutant Loading Rates, and Table 1 of §503.13. Ceiling Concentrations, US EPA CFR Part 503 (Biosolids Rules, 7–1–99 Edition).

2. Referenced Documents

2.1 *TMECC Sections:*

Section 04.12 Digestion Techniques

Section 04.13 Atomic Absorption Spectrometry

Section 04.14 Inductively Coupled Plasma

2.2 *Other References:*

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3. Terminology

3.1 *arsenic, n*—(symbol As) A highly poisonous metallic element having three allotropic forms, yellow, black, and gray, of which the brittle, crystalline gray is the most common. Arsenic and its compounds are used in insecticides, weed killers, solid-state doping agents, and various alloys. Atomic number 33; atomic weight 74.922; valence 3, 5. Gray arsenic melts at 817°C (at 28 atm pressure), sublimes at 613°C, and has a specific gravity of 5.73.

3.2 *beryllium, n*—(symbol Be) A high-melting, lightweight, corrosion-resistant, rigid, steel-gray metallic element used as an aerospace structural material, as a moderator and reflector in nuclear reactors, and in a copper alloy used for springs, electrical contacts, and nonsparking tools. Atomic number 4; atomic weight 9.0122; melting point 1,278°C; boiling point 2,970°C; specific gravity 1.848; valence 2.

3.3 *cadmium, n*—(symbol Cd) A soft, bluish-white metallic element occurring primarily in zinc, copper, and lead ores, that is easily cut with a knife and is used in low-friction, fatigue-resistant alloys, solders, dental amalgams, nickel-cadmium storage batteries, nuclear reactor shields, and in rustproof electroplating. Atomic number 48; atomic weight 112.40; melting point 320.9°C; boiling point 765°C; specific gravity 8.65; valence 2.

3.4 *copper, n*—(symbol Cu) A ductile, malleable, reddish-brown metallic element that is an excellent conductor of heat and electricity and is widely used for electrical wiring, water piping, and corrosion-resistant parts, either pure or in alloys such as brass and bronze. Atomic number 29; atomic weight 63.54; melting point 1,083°C; boiling point 2,595°C; specific gravity 8.96; valence 1, 2.

3.5 *iron, n*—(symbol Fe) A silvery-white, lustrous, malleable, ductile, magnetic or magnetizable, metallic element occurring abundantly in combined forms, notably in hematite, limonite, magnetite, and taconite, and used alloyed in a wide range of important structural materials. Atomic number 26; atomic weight 55.847; melting point 1,535°C; boiling point 2,750°C; specific gravity 7.874 (at 20°C); valence 2, 3, 4, 6.

3.6 *lead, n*—(symbol Pb) A soft, malleable, ductile, bluish-white, dense metallic element, extracted chiefly from galena and used in containers and pipes for corrosives, solder and type metal, bullets, radiation shielding, paints, and antiknock compounds. Atomic number 82; atomic weight 207.19; melting point 327.5°C; boiling point 1,744°C; specific gravity 11.35; valence 2, 4.

3.7 *manganese, n*—(symbol Mn) A gray-white or silvery brittle metallic element, occurring in several allotropic forms, found worldwide, especially in the ores pyrolusite and rhodochrosite and in nodules on the ocean floor. It is alloyed with steel to increase strength, hardness, wear resistance, and other properties and with other metals to form highly ferromagnetic materials. Atomic number 25; atomic weight 54.9380; melting point 1,244°C; boiling point 1,962°C; specific gravity 7.21 to 7.44; valence 1, 2, 3, 4, 6, 7.

3.8 *mercury, n*—(symbol Hg) A silvery-white poisonous metallic element, liquid at room temperature and used in thermometers, barometers, vapor lamps, and batteries and in the preparation of chemical pesticides. Atomic number 80; atomic weight 200.59; melting point -38.87°C; boiling point 356.58°C; specific gravity 13.546 (at 20°C); valence 1, 2.

3.9 *nickel, n*—(symbol Ni) A silvery, hard, ductile, ferromagnetic metallic element used in alloys, in corrosion-resistant surfaces and batteries, and for electroplating. Atomic number 28; atomic weight 58.71; melting point 1,453°C; boiling point 2,732°C; specific gravity 8.902; valence 0, 1, 2, 3.

3.10 *selenium, n*—(symbol Se) Used in pigments, electronics, xerography, and photographic exposure meters; a principal industrial use is for photvoltaic and photoconductive purposes. The color of the mineral form is reddish-gray to red. Atomic number 34; atomic weight 78.96; melting point 217.0°C; boiling point 684.9°C.

3.11 *standard, n*—Serving as or conforming to a standard of measurement or value. Sample often referred to a standard reference sample or check of known physical, chemical or biological characteristics used to monitor analytical bias or accuracy of a physical, chemical or biological determination.

3.12 *strontium, n*—(symbol Sr) A soft, silver-yellow alkaline-earth metal; strontium-90 from nuclear fallout occurs in plants and animals, and is linked with and may induce bone cancer and leukemia. The concentration of strontium is ranked as 15th out of 103 elements with respect to its abundance in the Earth's crust. Atomic number 38; atomic weight 87.62; melting point 769.0°C; boiling point: 1384.0°C.

3.13 *vanadium, n*—(symbol V) A soft, silver-gray metal used to add strength and heat resistance to steel

alloys; used in ceramics, glass, and dyes; important as catalysts in the chemical industry. Atomic number 23; atomic weight 50.9415; melting point 1890.0°C; boiling point 3380.0°C.

3.14 *zinc, n*—(symbol Zn) A bluish-white, lustrous metallic element that is brittle at room temperature but malleable with heating. It is used to form a wide variety of alloys including brass, bronze, various solders, and nickel silver, in galvanizing iron and other metals, for electric fuses, anodes, and meter cases, and in roofing, gutters, and various household objects. Atomic number 30; atomic weight 65.37; melting point 419.4°C; boiling point 907°C; specific gravity 7.133 (25°C); valence 2.

Test Method: Heavy Metals and Hazardous Elements. Arsenic							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-As	04.06-As						04.06-As	

04.06-AS ARSENIC

4. Significance

4.1 Arsenic is a trace element considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost. Arsenic is a human carcinogen and neurotoxin. Arsenic found in compost is usually far below levels of concern.

5. Selection of Method

5.1 *Digestion:*

5.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

5.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

5.1.3 Method 04.12-E—Aqua Regia Procedure.

5.2 *Determination:*

5.2.1 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Beryllium							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Be	04.06-Be						04.06-Be	

04.06-BE BERYLLIUM

6. Significance

6.1 Beryllium is a transition metal and is not required for plant growth. Beryllium is not considered to be a potential pollutant by the US EPA and the USDA.

7. Selection of Method

7.1 *Digestion:*

7.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

7.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

7.1.3 Method 04.12-E—Aqua Regia Procedure.

7.2 *Determination:*

7.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

7.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Cadmium							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Cd	04.06-Cd						04.06-Cd	

04.06-CD CADMIUM

8. Significance

8.1 Cadmium is a trace element considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost. Cadmium is readily taken up and translocated by plants, especially leafy green vegetables such as lettuce and spinach. If ingested as a result of plant uptake, cadmium can be a human health concern.

9. Selection of Method

9.1 *Digestion:*

9.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

9.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

9.1.3 Method 04.12-E—Aqua Regia Procedure.

9.2 *Determination:*

9.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

9.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Copper							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Cu	04.06-Cu						04.06-Cu	04.05-Cu

04.06-CU COPPER

10. Significance

10.1 Copper is a transition metal considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost. Copper in compost generally poses no human health risk. Copper is readily taken up and translocated by plants, however, plant health is severely compromised at concentrations below those toxic to humans. Copper can cause phytotoxicity, and can be an animal health concern through direct ingestion.

11. Selection of Method

11.1 *Digestion:*

11.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

11.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

11.1.3 Method 04.12-E—Aqua Regia Procedure.

11.2 *Determination:*

11.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

11.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Chromium							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Cr	04.06-Cr						04.06-Cr	

04.06-CR CHROMIUM

12. Significance

12.1 Chromium is a transition metal considered by the US EPA to be a potential pollutant. According to the USDA, as reported by the W-170 Peer Review Committee, Cr in biosolids and biosolids compost is Cr^{+3}, and no experimental evidence was reported that biosolids Cr causes adverse effects to any component of the ecosystem, and concluded that biosolids Cr limits be deleted from the Part 503 rule. Cr^{+3} poses no known human health risk. Phytotoxicity poses the greatest environmental risk, although chromium is not readily taken up and translocated by plants. Cr^{+6} poses known human health risk.

13. Selection of Method

13.1 Digestion:

13.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

13.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

13.1.3 Method 04.12-E—Aqua Regia Procedure.

13.2 *Determination:*

13.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

13.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Lead						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Pb	04.06-Pb						04.06-Pb	

04.06-PB LEAD

14. Significance

14.1 Lead is a transition metal considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost. Lead is strongly bound in all types of soil. Lead taken up by plants is strongly immobilized in the roots and is not readily translocated to above ground plant parts. Direct consumption of compost products by humans presents the greatest environmental risk from lead in composts. This exposure pathway is most common among children.

15. Selection of Method

15.1 *Digestion:*

15.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

15.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

15.1.3 Method 04.12-E—Aqua Regia Procedure.

15.2 *Determination:*

15.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

15.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Mercury						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Hg	04.06-Hg						04.06-Hg	

04.06-HG MERCURY

16. Significance

16.1 Mercury is a trace metal considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost; the pollutant concentration limit is 17 mg kg^{-1} dw. Mercury is not readily taken up by plants as it is strongly bound in the soil. Mercury that is taken up by plants is immobilized in the roots and is not significantly translocated to the above ground plant portions. Mercury is a human health concern, (e.g., when ingested as a result of uptake by certain kinds of edible mushrooms). Mercury will accumulate and is sequestered in the hypothalmus of the human brain. Landscape runoff containing mercury contaminated compost may reach sensitive wetland environments where it can be converted to methylmercury and accumulate in the aquatic food chain.

17. Mercury Determinations Review

17.1 *Drying of Biological Samples*—Although the US EPA suggests drying sediment at 60°C with no loss in mercury, others have observed mercury losses at temperatures exceeding 40°C. Iskandar, (1972), recovered essentially quantitative amounts of spiked mercury from soil and lake sediments dried at 60°C for 16 hours; but methyl mercury chloride and phenyl mercury hydroxide showed 16.8% and 11.7% losses as compared to 2.3% and 0.2% losses respectively from phenyl mercury acetate and Hg^{++}.

17.2 *Storage of Compost Samples*—Storage of a milled and air-dried (36°C) MSW compost sample at room temperature (~27°C) in sealed containers for approximately one year showed no apparent losses.

17.3 *Preservation and Storage of Sample Digests, Water Samples, and Calibration Standards*—Litman, et al., (1975) inferred that mercury losses in dilute solutions are due to the adsorption of reduced mercury (Hg^0) on the surfaces of storage vessels. A comparison showed that sorption was highest for polyethylene, lowest for Teflon and intermediate for glass. Strong oxidizing agents, (e.g., dichromate, or tetrachloraurate (III)) maintain mercury as Hg^{++} preventing sorption. Experiments conducted in glass vessels showed that sorption was a function of time from both, Hg in deionized water and in 1 *N* nitric acid. Sorption sites were finite so that at concentrations above 1 µg mL^{-1}, losses are insignificant.

17.4 *Digestion of Samples for Mercury Determinations.*

17.4.1 *Digestion of Soil and Rock Samples*—Procedures for digestion of soils, sediments and rock generally employ potassium permanganate in addition to acids and peroxide to break down the more stable metallic organic compounds. A sodium chloride-hydroxylamine hydrochloride reagent is also generally used to eliminate any interference with chloride. For metals, rocks and soils from 0.5 to 1 g samples are dissolved in sulfuric and nitric acid, with organic matter oxidized by hydrogen peroxide and potassium permanganate. A sodium chloride-hydroxylamine solution is used to reduce any excess oxidizing agent and keep the mercury as Hg^{++}. A 10% solution of stannous chloride, or stannous sulfate, is used to reduce mercury to Hg. In some instances, potassium persulfate was shown to be more effective than potassium permanganate in oxidizing organo-mercury in soil, and Melton et. al., (1972) obtained 97 to 102.5% recovery in soils (0.5 g samples) by using 10 mL of saturated $K_2S_2O_8$ with 10 mL each of concentrated nitric and sulfuric acid for a 1 h digestion at room temperature (~27°C). Twenty mL of a reducing solution containing 20 g NaCl, 20 g hydroxylamine hydrochloride, 33 g of $SnCl_2$, 1 g of hydrazine sulfate, and 9 mL of sulfuric acid per liter was used to reduce mercury and oxidizing agents. Iskandar (1972) prevented mercury loss by digesting 1 g of soil or lake sediments with 15 mL of a 2:1 mixture of sulfuric and nitric acids at 50 to 60°C for 2 to 3 hours. Use of nitric acid alone was effective for most samples, except those particularly high in organic matter. It was concluded that the use of both sulfuric and nitric acid effectively breaks down organic matter found in soil and lake sediments; and any partially oxidized organic matter that is present after digestion. Digestion with nitric acid alone caused foaming during aeration and low mercury recovery. Iskandar (1972) suggested that plastic ware be avoided because of its tendency to sorb mercury, and that an additional 10 mL of acid be used for samples high in organic matter. After cooling the cleared digest, it is placed in an ice bath where excess 5% potassium permanganate is added (5 mL or more), followed by 2 mL of 5%

potassium persulfate. The reactants should be left for 4 h or preferably overnight at room temperature (~27°C) to insure the oxidation of organomercury as recommended by EPA. Also, the use of an ice bath when adding the initial acids is recommended to prevent the volatilization and loss of mercury. They conclude that erroneous results may occur if potassium permanganate is not added to prevent volatilization of mercury prior to reduction by stannous chloride. Three mL of 20% tin chloride (60 mg) in 6 N HCl was necessary to insure reduction of excess potassium permanganate and mercury.

17.4.2 *Digestion of Biological Samples for Mercury with Emphasis on Microwave Methods*—Many different acid or acid mixtures have been used, including various combinations of nitric, sulfuric and hydrochloride acids, often followed by oxidation with potassium permanganate or peroxidisulfate to insure complete recovery of methyl or alkyl mercury in soil, sediments and water samples, and fish. Procedures developed after Melton et al. (1971) suggest the use of 10 mL of 1:1 nitric and sulfuric acid with 5 mL of saturated potassium persulfate (35 g of $K_2S_2O_8$ in 500 mL of water) for 0.5 to 1.0 g of soil or 200 mg of plant tissue. The high volatility and mobility of mercury is a problem and many showed that closed vessels are necessary to prevent mercury loss during digestion. Others use elaborate condensation traps and fluxing to prevent mercury loss. Van Delft and Vos (1988) concluded that closed vessels, either a Teflon bomb heated in a conventional oven or digestion in a microwave oven, were necessary for complete recovery of mercury in soils and sediments.

17.4.2.1 In their microwave method, Van Delft and Vos (1988) used 6 mL of concentrated HCl and 2 mL of concentrated nitric acid with 10 mL of water and 0.5 g of soil or sediment, without using any permanganate or peroxidisulfate. For the digestion of 100 mg biological samples (NBS milk, oyster, wheat, bovine liver samples), Vermir, et al. (1989) used 1 mL of concentrated nitric acid. Krechtel and Fraser (1989) noted that the high carbon content of biological samples acts as a strong reducing agent that leads to volatilization and loss of mercury and used 13 mL of 0.25 *N* potassium permanganate per L of standard sample.

17.4.2.2 Microwave time and power settings vary. Vermeir et al., (1989) used 8 min at 20% power, 8 min at 40% power and 4 min at 60% power; while Van Deft and Vos, (1988) use settings of 30% for 1 min, 80% for 4 min and 100% for 10 min.

17.4.2.3 There is some debate about the type of vessel suitable for microwave digestion. Tatro concluded that a typical closed vessel for microwave digestion constructed of PFA Teflon is not adequate for mercury digestion because mercury vapor tends to absorb through the Teflon walls. He recommended open vessel digestion or the use of Prolab brand microwave digestion system with vessels constructed of quartz to prevent the loss of mercury vapor. However, losses from sorption are lowest for Teflon, highest for polyethylene and intermediate for glass (Litman, et al. 1975).

17.4.2.4 Losses during wet digestion are as high as 30%. Litman, et al. (1975) concluded that an important contributing factor is the dilution of mercury, because insignificant losses in an initially high mercury concentration become significant when it is diluted 100x in the digestion procedure. In addition, considerable mercury may be introduced when large quantities of reagents are added, (e.g., permanganate may contain mercury). As previously noted, potassium permanganate and sodium chloride-hydroxylamine solution are used to reduce excess permanganate.

17.4.3 *Reduction of Mercury in Samples for Cold Vapor AA Analysis*—The reduction of mercury is relatively simple, quite sensitive and applicable to a wide variety of samples with appropriate pretreatment. Digestions are designed to convert all the mercury to Hg^{++}, which is then reduced to Hg by addition of Sn_2Cl. The mercury is then vaporized and passed through the atomic absorption spectrophotometer (AA) absorption cell.

NOTE HG1—Alkymercuricals are generally determined by electron capture gas chromatography.

17.4.3.1 Either tin chloride ($SnCl_2$) or tin sulfate ($SnSO_4$) is used to reduce metal mercury to the gaseous form for analysis. Chloride is preferred because it is more soluble, while sulfate remains in suspension and requires constant stirring. Tin chloride needs to be made up frequently; weekly replacement is suggested by some. Also the concentration of $SnCl_2$ used in the reduction process varies widely. Vermeir et al., (1989) add 1 mL of a 30% solution or 300 g for their digest from 100 mg of biological tissue, while Van Delft and Vos, (1988) add 0.5 mL of a 50% $SnCl_2$ solution or 100 mg for their digest from 0.50 g of soil. Procedures from the US EPA use 10 mL of a 10% stannous chloride ($SnCl_2$) solution or 1000 g for 0.5 g of sediment or mL of water. The Minnesota Dept. of Health recommends the use of 1 mL of a 20% $SnCl_2$ for a 40-mL water sample or 200 mg of $SnCl_2$. The method from the manufacturer of LCD mercury monitor instrument as described, recommends the addition of 6 mL of 10% $SnCl_2$ solution or 600 mg $SnCl_2$ for standards made with 10 % HCl solution. In contrast US EPA Method 7471A calls for an addition of 0.05 mL of 10% $SnCl_2$ or $SnSO_4$ for each mL of digest (e.g., 0.25 mL for the 5-mL digests). It was found that most mercury is reduced and volatized in the reaction vessel by bubbling nitrogen gas alone, and that

0.25 mL of $SnCl_2$ insures a rapid reduction and response in the LCD mercury monitor.

17.4.3.2 Since $SnCl_2$ only reduces metal mercury, several other chemicals were used to reduce organic and ethyl mercury to the gaseous form for analysis by cold vapor AA. Sodium borohydride reduces both inorganic and organic mercury, but iodide, selenium and $NaBH_4$ interfere in the analysis (de Vargis and Romero 1989). Antonovich, et al., 1991, demonstrated that hydrazine borane (HB) could be used to selectively reduce phenyl and methyl organic mercury. HB is a stronger reducing agent (-0.30 V) than $SnCl_2$ (+0.15 V) and the latter does not reduce organic mercury under acidic conditions. HB reduces Hg^{++} in organic compounds in acidic and alkaline media, but it reduces mercury in phenyl and alkyl compounds only in acidic media. $NaBH_4$ reduces both alkyl and phenyl compounds vigorously in both acid and alkaline solutions.

17.4.3.3 An instrument note by Leeman Labs, Lowell, Mass. suggests that permanganate be generally used to insure breakdown of metallo-organics with $K_2S_2O_8$ (potassium persulfate) assuring the oxidation of stable organic mercuricals. Free chlorine formed with the addition of permanganate, absorbs light at the 254-nm wavelength, and mercurous chloride may precipitate as Hg_2Cl_2 and lead to low results. The addition of sodium chloride-hydroxylamine sulfate reduces the free Cl_2 levels to insure that it will not interfere with the UV absorption or consume permanganate. High chloride is a problem with sea water, brines, and certain effluents. Some use gold wire, or gold-coated glass frit or sand to concentrate the mercury, that is later released to the absorption cell by the application of heat to the collection media. The ideal dead volume should be smaller than cell volume, so the total sample can be in the light beam all at once. Klein (1972) generally observed high blanks for the first few runs each day, presumably due to mercury condensing in the air train or glassware, but low (a few ng) blanks are obtained after a few runs.

17.4.4 In the Research Analytical Laboratory, University of Minnesota, the digestion and preparation of a 0.5 mL aliquot from the compost digestion (US EPA 3051) for cold vapor atomic adsorption analysis is conducted in a 50 mL polyethylene (Evergreen) centrifuge tube fitted with a screw top (US EPA Method 7470A). The compost digestion (US EPA Method 3051) results in a 25 % nitric acid solution; and 0.5 mL of this digest solution is first diluted to 5 mL to obtain the correct concentration of nitric acid (2.5 %). Sulfuric acid, potassium permanganate and potassium persulfate are then added, and the mixture is heated at 95°C for 2 h in a water bath. Since gaseous mercury can diffuse through the walls of polyethylene containers, the sample is cooled and retained in the acid/permanganate/persulfate mixture to maintain mercury in the inorganic form and minimize loss. Hydroxylamine is not added to the vessel until cold vapor AA mercury analysis is conducted. This analysis must be conducted within one day of the oxidation. At this time, the 50-mL polyethylene centrifuge tube fitted with a 3-hole stopper is attached to cold vapor AA apparatus and $SnCl_2$ is added to reduce the inorganic mercury to volatile gaseous mercury. The Hg^0 vapor is then flushed into the atomic absorption spectrophotometer (MercuryMonitor™) and the absorbance recorded. The use of the 50-mL centrifuge tube for both the digestion and reduction steps eliminates the need for a transfer step in which mercury could be lost. Any inorganic mercury sorbed to the vessel is reduced and flushed into the analyzer without loss.

17.4.5 *Desiccation of the Nitrogen Stream*—Some difficulty was observed with the magnesium perchlorate ($Mg(ClO_4)_2$) drying tube employed in the cold vapor technique for mercury determination. As the drying tube picks up water vapor, it impedes the gas flow, suppresses peaks and slows recovery time. A sulfuric acid desiccator that eliminated spurious absorbency due to moisture, and the drawbacks of the $Mg(ClO_4)_2$ drying tube was developed (Kotanadaraman and Dallmeyer, 1976). When analyzing large numbers of samples with this desiccator, the sulfuric acid should be agitated periodically to avoid saturating the surface layer. Melton et al., (1971) used an ice bath condenser to dry the nitrogen stream rather than a desiccant. EPA methods suggest replacing the drying tube of magnesium perchlorate with a 60 watt incandescent bulb, and to heat the cell to 10°C above ambient room temperature (~27°C) to eliminate interference from water vapor. A comparison of these strategies at the Research Analytical Laboratory, University of Minnesota, showed that a combination of a condensation trap in tandem with a drying tube filled with anhydrous $Mg(ClO_4)_2$ proved most effective in removing water vapor from the nitrogen stream of the cold vapor AA.

17.4.6 *Detection Limits Observed and sample size Cold Vapor AA*—To obtain a sensitivity of 10 ppb, 1 g of a sample containing 0.01 µg of mercury is required (Isklander 1972). 1 g of soil or lake sediment was adequate to obtain a standard deviation of 0.033 mg kg^{-1} with a concentration of 1.61 mg kg^{-1}.

17.4.6.1 Manning (1970) noted that the detection limits for the cold vapor AA are of the order of 0.001 µg or lower in solution. Organics, including cyclic organics, sulfides, and water vapor, interfere with the analysis

Table 04.06-Hg1 Background levels of mercury in non-contaminated samples from various sources.

Sample	*Hg (ppb or μg L^{-1})*
Air	0.002
Surface Water	0.05 (Lake Michigan)
Sea water	0.1
Rain water	0.15
Raw sewage	2
Crustal rocks	50
Soils and sediments	50
Coal	200
Fish	100 (2 mg kg^{-1})
Man	100
Biosolids	500

ADAPTED FROM—Klein, 1972.

18. Selection of Method

18.1 US EPA Method 7471A is the solid waste (SW 486) method recommended for mercury determinations in solid or semi-solid wastes; while US EPA Method 3051 (microwave assisted) is recommended for the acid digestion of sediments, biosolidss, soils, and oils in preparation for metal analysis, including mercury. US EPA Method 3051 is considered advantageous for compost analysis because it employs a closed microwave vessel that prevents the loss of mercury by volatilization during digestion (Van Delft and Vos 1988.; Vermir, et al. 1989). Such losses are likely with composts that contain organic matter levels higher than in most soils and sediments (Iskandar 1972; Krechtel and Fraser 1979). The US EPA 3051 method is also advantageous because it prepares the compost sample for the analysis of a wide variety of metals in addition to mercury. The SW-486 US EPA Method 7070A, designed for the mercury analysis of liquid waste can then be used to prepare an aliquot of the 3051 digest for cold vapor AA mercury analysis. Cold vapor AA analysis has a mercury detection level far below that for ICP-AES analysis. The digestion method, US EPA Method 7470A is considered more rigorous than the US EPA 7471A method because it employs persulfate in addition to permanganate. Persulfate is important to insure the dissolution of metallo-mercury complexes, including methyl mercury and is important for the complete dissolution of mercury in soil digestions (Melton et al., 1971). Furthermore, the use of the 7470A method is necessary for preparing the sample for Cold Vapor AA analysis.

18.2 *Digestion:*

18.2.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

18.3 *Determination:*

18.3.1 Method 04.13-A—Cold Vapor AAS Technique for Mercury in Compost.

Test Method: Heavy Metals and Hazardous Elements. Molybdenum							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Mo	04.06-Mo						04.06-Mo	04.05-Mo

04.06-MO MOLYBDENUM

19. Significance

19.1 Molybdenum is considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost. Molybdenum can be a human health concern through direct ingestion, and an animal health concern through direct ingestion or through ingestion of forage with high molybdenum concentrations grown in inland desert areas.

19.2 Molybdenum is an essential trace element for plant growth. It is a constituent of proteins, especially enzymes. It is involved in symbiotic N_2 fixation, nitrate reduction. It is a constituent of the enzymes: nitrate reductase, nitrogenase, oxidases and the cofactor: molybdoferredoxin. and is found in organelles such as mitochondria.

19.3 Molybdenum is taken up by plants as the molybdate ion, MoO_4^{2}. It is required by plants to transform nitrate into amino acids; plants cannot transform nitrogen into amino acids without molybdenum. Legumes cannot fix atmospheric nitrogen symbiotically unless molybdenum is present. Molybdenum was found in some regions of the country at levels toxic to livestock due to high concentrations in forage. Deficiencies of molybdenum have required small additions to legumes grown in other regions.

19.4 Deficient concentrations in agronomic crop plant leaf tissues range from 0.1 mg kg^{-1} to 0.3 mg kg^{-1}. Sufficient concentrations in agronomic crop plant leaf tissues range form 0.02 to 5.0 mg kg^{-1}, up to a tolerable concentration less than 10.0 mg kg^{-1}. The common deficiency symptom is chlorosis of leaf margins. This is due to excessive accumulation of nitrate that in turn causes destruction of embryonic tissues. Excessive and toxic concentrations range from 10-50 mg kg^{-1}.

20. Selection of Method

20.1 *Digestion:*

20.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

20.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

20.1.3 Method 04.12-E—Aqua Regia Procedure.

20.2 *Determination:*

20.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

20.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Nickel						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Ni	04.06-Ni						04.06-Ni	

04.06-NI NICKEL

21. Significance

21.1 Nickel is a transition metal and is not required for plant growth. Nickel is considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost. Nickel at levels usually found in compost does not pose a risk to the food chain. Phytotoxicity of plants poses the greatest environmental risk.

22. Selection of Method

22.1 *Digestion:*

22.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

22.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

22.1.3 Method 04.12-E—Aqua Regia Procedure.

22.2 *Determination:*

22.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

22.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Selenium						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Se	04.06-Se						04.06-Se	

04.06-SE SELENIUM

23. Significance

23.1 Selenium is a trace element that is generally nontoxic in the elemental form and is considered essential; other forms, including hydrogen selenide, are extremely toxic and cause similar physiological reactions to those of arsenic. Selenium is considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost. Selenium can be a human or animal health concern through consumption of plants that contain excessive concentrations of selenium; hydrogen selenide concentrations greater than 1.5 mg kg^{-1} are toxic to man. In certain regions in the western USA, soils are naturally enriched in selenium and plants grown on the sites become enriched as well.

24. Selection of Method

24.1 *Digestion:*

24.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

24.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

24.2 *Determination:*

24.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

24.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Strontium							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Sr	04.06-Sr						04.06-Sr	

04.06-SR STRONTIUM

25. Significance

25.1 Strontium-90 from nuclear fallout occurs in plants and animals, and is linked with, and may induce bone cancer and leukemia. Upon ingestion by mammals, strontium is distributed in three body compartments: plasma extracellular fluid; soft tissue and superficial zone of bone tissue; and bone itself. The average adult human is estimated to have a body burden of 320 mg strontium, 99% of which is in the bones. The toxic effect of excessive strontium intakes is inhibition of calcification of epiphyseal cartilage and deformities of long bones at high doses. Strontium causes adverse effects on bone by substituting for calcium in the hydroxyapatite crystal during bone calcification or by displacing calcium from existing calcified matrix.

26. Selection of Method

26.1 *Digestion:*

26.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

26.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

26.1.3 Method 04.12-E—Aqua Regia Procedure.

26.2 *Determination:*

26.2.1 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Vanadium							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-V	04.06-V						04.06-V	

04.06-V VANADIUM

27. Significance

27.1 Vanadium is ranked as one of the most hazardous compounds (worst 10%) to human health.

28. Selection of Method

28.1 *Digestion:*

28.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

28.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

28.1.3 Method 04.12-E—Aqua Regia Procedure.

28.2 *Determination:*

28.2.1 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Heavy Metals and Hazardous Elements. Zinc						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.06-Zn	04.06-Zn						04.06-Zn	04.05-Zn

04.06-ZN ZINC

29. Significance

29.1 Zinc is a trace metal considered to be a potential pollutant by the US EPA and the USDA. It is regulated by the US EPA at 40 CFR Part 503 in biosolids and biosolids compost.

29.2 Zinc is an essential micronutrient for plant growth. It is an essential constituent of several important enzyme systems in plants. It controls the synthesis of indoleactetic acid, an important plant growth regulator. It is bound into miscellaneous large molecules combined with proteins, including enzymes, and found in organelles such as mitochondria. It is involved in carbohydrate, nucleic acid and lipid metabolism. It is a constituent of anhydrases, dehydrogenases, proteinases, and peptides.

29.3 Deficient concentrations in agronomic crop plant leaf tissues range from 10 mg kg^{-1} to 20 mg kg^{-1}. Sufficient concentrations in agronomic crop plant leaf tissues range from 27 mg kg^{-1} to 150 mg kg^{-1}, up to a commonly tolerable concentration less than 300 mg kg^{-1}. Excessive and toxic concentrations range from 100-400 mg kg^{-1}.

29.4 Terminal growth areas are affected first when zinc is deficient. Zinc is taken up by plants as the divalent ion (Zn^{++}). The common deficiency symptom is interveinal chlorosis in monocots, stunted growth and violet-red points on leaves. Zinc is the micronutrient most often needed by crops in some regions of the USA.

30. Selection of Method

30.1 *Digestion:*

30.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

30.1.2 Method 04.12-B—Nitric Acid Digestion, SW-846 US EPA 3050B Modified.

30.2 *Determination:*

30.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy.

30.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

04.06 METHODS SUMMARY

31. Report

31.1 Correct all metals on an oven-dried weight basis (forced-air oven dried at 70±5°C) of the compost or feedstock sample. Report the digest method and determination technique used for the test.

31.2 Report in parentheses the concentration reported as presented in equation 31.2.1 corrected to 30% LOI organic matter content.

31.2.1 *Standardize to 30% Organic Matter Content:*

$$E' = E \times OM \div 30 \qquad \textit{Equation 31.2.1}$$

where:

E' = standardized relative elemental concentration,
E = oven-dried weight basis for elemental concentration, mg kg^{-1},
30 = relative percentage organic matter of a stabilized and mature compost, %, and
OM = loss-on-ignition organic matter content of the compost sample, %.

31.3 *Reporting Units*—Report results as mg kg^{-1} dw.

32. Precision and Bias for Methods

32.1 Precision for the following tests were determined using ICP-AES and US EPA 3051 modified for compost by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN.

32.1.1 *Precision*—Determined for 10 subsamples taken from a field composite sample for each of three sites at two separate sampling periods (1993). Variability is expressed as standard deviation (Std Dev) and percent coefficient of variation (%CV).

32.2 *Method 04.06-Arsenic, mg kg^{-1}:*

Table 04.06-Ar1 Intra-sample precision from three sites and two sample dates for arsenic.

Median	*Std Dev*	*% CV*	*Number of Samples*
10.1	0.5	4.7	10
6.2	1.4	22.3	10
12.1	1.1	9.0	10
8.3	0.6	7.2	10
10.7	0.7	6.7	10
7.3	1.0	14.4	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

32.3 *Method 04.06-Cd Cadmium, mg kg^{-1}:*

Table 04.06-Cd1 Intra-sample precision from three sites and two sample dates for cadmium.

Median	*Std Dev*	*% CV*	*Number of Samples*
14.6	0.8	5.5	10
10.7	0.3	2.6	10
10.8	12.0	82.5	10
11.9	3.8	28.8	10
7.5	0.6	8.1	10
7.6	0.8	10.7	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

32.4 *Method 04.06-Cu Copper, mg kg^{-1}:*

Table 04.06-Cu1 Intra-sample precision from three sites and two sample dates for copper.

Median	*Std Dev*	*% CV*	*Number of Samples*
441	34.2	7.7	10
342	13.7	3.9	10
754	172.0	20.8	10
788	103.4	12.9	10
296	65.5	20.4	10
316	15.0	4.7	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

32.5 *Method 04.06-Cr Chromium, mg kg^{-1}:*

Table 04.06-Cr1 Intra-sample precision from three sites and two sample dates for chromium.

Median	*Std Dev*	*% CV*	*Number of Samples*
73	8.7	11.7	10
66	6.7	9.7	10
86	7.4	8.5	10
112	8.6	7.5	10
57	34.6	45.6	10
65	10.6	16.0	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

32.6 *Method 04.06-Pb Lead, mg kg^{-1}:*

Table 04.06-Pb1 Intra-sample precision from three sites and two sample dates for lead.

Median	*Std Dev*	*% CV*	*Number of Samples*
360	16.8	4.6	10
268	11.1	4.1	10
402		9.4	10
403	41.9	10.0	10
296	9.9	3.4	10
320	17.8	5.5	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

32.7 *Method 04.06-Hg Mercury, mg kg^{-1}:*

Table 04.06-Hg2 Intra-sample precision from three sites and two sample dates for mercury.

Median	*Std Dev*	*% CV*	*Number of Samples*
2.6	0.23	8.9	10
2.2	0.13	5.7	9
7.7	0.64	8.3	10
6.1	0.39	6.3	10
6.5	0.60	9.0	10
7.1	0.58	8.1	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.12-A

32.8 *Method 04.06-Mo Molybdenum, mg kg^{-1}:*

Table 04.06-Mo1 Intra-sample precision from three sites and two sample dates for molybdenum.

Median	*Std Dev*	*% CV*	*Number of Samples*
6.16	0.26	4.2	10
6.41	0.81	12.0	10
7.85	0.20	2.5	10
6.40	0.41	6.4	10
5.55	1.00	17.1	10
5.47	0.39	7.1	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

32.9 *Method 04.06-Ni Nickel, mg kg^{-1}:*

Table 04.06-Ni1 Intra-sample precision from three sites and two sample dates for nickel.

Median	*Std Dev*	*% CV*	*Number of Samples*
72.5	5.5	7.6	10
59.1	8.1	13.1	10
61.9	5.1	8.1	10
75.9	4.0	5.2	10
55.3	17.5	27.8	10
55.7	4.7	8.4	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

32.10 *Method 04.06-Zn Zinc, mg kg^{-1}:*

Table 04.06-Zn1 Intra-sample precision from three sites and two sample dates for zinc.

Median	*Std Dev*	*% CV*	*Number of Samples*
909	32	3.5	10
785	19	2.5	10
1742	347	18.9	10
1784	254	13.5	10
1299	114	8.5	10
1436	102	7.0	10

NOTE—Digestion : Method 04.12-A; Determination : Method 04.14-A

32.11 *Method 04.06-Se Selenium*—Precision and bias for this test are not determined. Data are being sought to construct a precision and bias statement.

32.12 *Method 04.06-Sr Strontium*—Precision and bias for this test are not determined. Data are being sought to construct a precision and bias statement.

32.13 *Method 04.06-V Vanadium*—Precision and bias for this test are not determined. Data are being sought to construct a precision and bias statement.

33. Keywords

33.1 heavy metal; contaminant; arsenic; beryllium; cadmium; chromium; copper; iron; lead; manganese; mercury; nickel; selenium; strontium; vanadium; zinc

Test Method: Other Elements. Six [6] Elements							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
Elements presented in this section are of minimal concern to compost producers, are not regulated and are generally not of interest to compost users.								

04.07 OTHER ELEMENTS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the determination of elements not considered to be potential pollutants by the US EPA and the USDA nor considered to be essential for plant growth.

1.1.1 *Method 04.07-Al Aluminum.*

1.1.2 *Method 04.07-Sb Antimony.*

1.1.3 *Method 04.07-Ba Barium.*

1.1.4 *Method 04.07-Cn Cyanides.*

1.1.5 *Method 04.07-Ag Silver.*

1.1.6 *Method 04.07-Tl Thallium.*

1.2 All methods and sampling protocols provided in TMECC are subject to revision and update to accommodate new widely accepted advances in techniques and methods.

2. Referenced Documents

2.1 *TMECC Sections:*

Section 04.12 Digestion Techniques

Section 04.13 Atomic Absorption Spectrometry

Section 04.14 Inductively Coupled Plasma

2.2 *Other References:*

US EPA Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. SW-846. 3rd Edition. 1992.

3. Terminology

3.1 *aluminum, n*—(symbol Al) A silvery-white, ductile metallic element, the most abundant in the earth's crust but found only in combination, chiefly in bauxite. Having good conductive and thermal properties, it is used to form many hard, light, corrosion-resistant alloys. Atomic number 13; atomic weight 26.98; melting point 660.2°C; boiling point 2,467°C; specific gravity 2.69; valence 3.

3.2 *antimony, n*—(symbol Sb) A metallic element having four allotropic forms, the most common of which is a hard, extremely brittle, lustrous, silver-white, crystalline material. It is used in a wide variety of alloys, especially with lead in battery plates, and in the manufacture of flame-proofing compounds, paint, semiconductor devices, and ceramic products. Atomic number 51; atomic weight 121.75; melting point 630.5°C; boiling point 1,380°C; specific gravity 6.691; valence 3, 5.

3.3 *barium, n*—(symbol Ba) A soft, silvery-white alkaline-earth metal, used to deoxidize copper and in various alloys. Atomic number 56; atomic weight 137.34; melting point 725°C; boiling point 1,140°C; specific gravity 3.50; valence 2.

3.4 *cyanide, n*—Any of various salts or esters of hydrogen cyanide containing a CN group, especially the extremely poisonous compounds, potassium cyanide and sodium cyanide.

3.5 *silver, n*—(symbol Ag) A lustrous white, ductile, malleable metallic element, occurring both uncombined and in ores such as argentite, having the highest thermal and electrical conductivity of the metals. It is widely used in coinage, photography, dental and soldering alloys, electrical contacts, and printed circuits. Atomic number 47; atomic weight 107.868; melting point 960.8°C; boiling point 2,212°C; specific gravity 10.50; valence 1, 2.

3.6 *thallium, n*—(symbol Tl) A soft, malleable, highly toxic metallic element, used in photocells, infrared detectors, low-melting glass, and formerly in rodent and ant poisons. Atomic number 81; atomic weight 204.37; melting point 303.5°C; boiling point 1,457°C; specific gravity 11.85; valence 1, 3.

Test Method: Other Elements. Aluminum						**Units:** % dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*

04.07-AL ALUMINUM

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

4. Significance

4.1 Aluminum is not considered to be a potential pollutant by the US EPA or the USDA. Aluminum at levels found in compost does not pose a risk to the food chain. Phytotoxicity poses the greatest environmental risk.

4.2 The phytotoxic effect of aluminum increases at pH values below 5.5. High concentrations of aluminum diminish root growth by inhibiting elongation. This reduces a plant's access to soil moisture and essential nutrients such as calcium. In addition, aluminum may directly inhibit uptake of phosphorus through precipitation of aluminum phosphate at the root surface.

5. Selection of Method

5.1 *Digestion:*

5.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

5.1.2 Method 04.12-B—Nitric Acid Digestion, US EPA 3050B Modified.

5.1.3 Method 04.12-C—Dry Ash Sample Digestion for Plant Nutrients.

5.1.4 Method 04.12-E—Aqua Regia Procedure.

5.2 *Determination:*

5.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy, or

5.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Other Elements. Antimony						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*

04.07-SB ANTIMONY

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

6. Significance

6.1 Antimony is not required for plant growth. Antimony is not considered to be a potential pollutant by the US EPA and the USDA.

7. Selection of Method

7.1 *Digestion:*

7.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

7.1.2 Method 04.12-B—Nitric Acid Digestion, US EPA 3050B Modified.

7.1.3 Method 04.12-E—Aqua Regia Procedure.

7.2 *Determination:*

7.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy, or

7.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Other Elements. Barium						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*

04.07-BA BARIUM

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Significance

8.1 Barium is not required for plant growth. Barium is not considered to be a potential pollutant by the US EPA and the USDA.

9. Selection of Method

9.1 *Digestion:*

9.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

9.1.2 Method 04.12-B—Nitric Acid Digestion, US EPA 3050B Modified.

9.2 *Determination:*

9.2.1 Method 04.14-A—Inductively Coupled Plasma - Atomic Emission Spectroscopy, or

9.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Other Elements. Cyanides						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*

04.07-CN CYANIDES

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

10. Significance

10.1 Cyanides are very poisonous to animals and humans and can cause death within seconds of inhalation or ingestion. Death is due to respiratory arrest. Cyanide is commonly found in certain rat and pest poisons, silver and metal polishes, photographic solutions, and fumigation products. Workers, such as electroplaters and picklers who are daily exposed to cyanide solutions may develop a "cyanide" rash, characterized by itching, and by macular, papular, and vesicular eruptions. Cyanides are also found in apricot, peach, and similar fruit pits and in sweet almonds, and in Laetrile, an alleged anti-cancer drug.

11. Selection of Method

11.1 *Digestion:*

11.1.1 US EPA Method 9013—Cyanide Extraction Procedures for Solids and Oils

11.2 *Determination:*

11.2.1 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Other Elements. Silver						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*

04.07-AG SILVER

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

12. Significance

12.1 Silver is a transition metal and is not required for plant growth. Silver is not considered to be a potential pollutant by the US EPA and the USDA.

13. Selection of Method

13.1 *Digestion:*

13.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

13.1.2 Method 04.12-B—Nitric Acid Digestion, US EPA 3050B Modified.

13.1.3 Method 04.12-E—Aqua Regia Procedure.

13.2 *Determination:*

13.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy, or

13.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

Test Method: Other Elements. Thallium						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*

04.07-Tl THALLIUM

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

14. Significance

14.1 Thallium is a transition metal and is not required for plant growth. Thallium is not considered to be a potential pollutant by the US EPA and the USDA.

15. Selection of Method

15.1 *Digestion:*

15.1.1 Method 04.12-A—Microwave-Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

15.1.2 Method 04.12-B—Nitric Acid Digestion, US EPA 3050B Modified.

15.1.3 Method 04.12-E—Aqua Regia Procedure.

15.2 *Determination:*

15.2.1 Method 04.14—Inductively Coupled Plasma - Atomic Emission Spectroscopy, or

15.2.2 Method 04.13-B—Atomic Absorption Spectrophotometry.

04.07 METHODS SUMMARY

16. Report

16.1 Correct all metals on an oven-dried weight basis (forced-air oven dried at 70±5°C) of the compost or feedstock sample. Report the digest method and determination technique used for each element. Report concentrations at three significant figures, mg kg^{-1}.

16.2 Report in parentheses, elemental concentrations as described in paragraph 16.1 corrected to 30% LOI organic matter content.

16.2.1 *Standardize to 30% Organic Matter Content:*

$$E' = E \times OM \div 30 \qquad \textit{Equation 16.2.1}$$

where:

E' = standardized elemental concentration,
E = oven-dried weight basis for elemental concentration, mg kg^{-1},
30 = relative percentage organic matter of a stabilized and mature compost, %, and
OM = loss-on-ignition organic matter content of the compost sample, %. Refer to Method 05.07-A.

17. Precision and Bias

17.1 Precision and bias are not determined for these elements. Data are being sought to formulate a precision and bias statement.

18. Keywords

18.1 aluminum; antinomy; barium; cyanides; silver; thallium; elements

Test Method: Inorganic Carbon. One Method.						**Units:** % $CaCO_3$		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.08-A						04.08-A		04.08-A

04.08 INORGANIC CARBON

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the measurement of total carbonates, the sum of carbonates and bicarbonates in compost.

1.1.1 *Method 04.08-A Calcium Carbonate Equivalency*—modified after AOAC 955.01, AS4454 and Methods of Soil Analysis, SSSA-ASA.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 02.02-E Milling and Grinding Samples, Munter

Method 03.08 Total Solids and Moisture

Method 04.01 Organic Carbon

3. Terminology

3.1 *acid, n*—a compound consisting of hydrogen plus one or more other elements which readily releases hydrogen when mixed with water or some solvents. Acids have pH values between 0 - 7. When an acid reacts with a base (alkali), a salt is formed. Acids act as corrosives, unless highly diluted.

3.2 *base, n*—an alkali; a compound with a pH value between 7.0 and 14.0. Bases react with acids to form a salt. Usually acts as a corrosive, unless it is highly diluted.

3.3 *carbonate, n*—ion, carbon trioxide, CO_3, molecular weight: 60.00935, CAS 3812-32-6.

4. Summary of Test Methods

4.1 A sample is treated with hydrochloric acid and the unreacted acid is back titrated against standard sodium hydroxide.

5. Significance and Use

5.1 Compost applications can affect changes in soil pH. Rating compost according to its % $CaCO_3$ equivalency can be used to approximate the relative magnitude of that change.

5.2 If present, carbonates not accounted for will inflate organic carbon determinations, i.e., Method 04.01 Organic Carbon.

6. Interference and Limitations

6.1 Extra care should be taken during the titration if the aliquot is dark. It may be difficult to differentiate a color change for the end point.

6.2 The magnitude of soil pH adjustment using compost will vary with soil condition. Field verification on the target soils must be performed prior to full-scale adoption of this method.

7. Sample Handling

7.1 Use an air-dried, milled sample. Store the prepared sample in a sealed container at ambient laboratory temperatures (approximately 23°C).

Test Method: Inorganic Carbon. Calcium Carbonate Equivalency						**Units:** % $CaCO_3$		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.08-A						04.08-A		04.08-A

04.08-A CALCIUM CARBONATE EQUIVALENCY

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

NOTE 1A—This method is not for reporting offical lime equivalency. The method gives approximate values which are viewed as meanigful and sufficenietly accurate enough for routine compost products.

8. Apparatus for Method A

8.1 *Extracting Bottle*—250-mL wide-mouth, plastic, (e.g., Nalgene or equivalent).

8.2 *Mechanical Shaker*—50 revolutions or excursions per minute.

8.3 *Filter Paper*—fluted.

8.4 *Centrifuge*—to spin at or more than 3000 rpm.

8.5 *Flask*—100-mL Erlenmeyer.

9. Reagents and Materials A

9.1 *Hydrochloric Acid*—1 *M* HCl.

9.2 *Sodium Hydroxide*—0.5 *M* NaOH.

9.2.1 Dissolve 20.0 g sodium hydroxide (NAOH pellets) in deionized water and bring to volume, 1.0 L. Standardize against potassium hydrogen phthalate. Take precaution to exclude carbon dioxide before standardization. Storage of NaOH should be short term, and re-standardized frequently.

9.3 *Phenolphthalein Indicator*—0.1%, dissolve 100 mg phenolphthalein in 100 mL ethanol.

9.4 *Calcium Carbonate*—powder, CAS 471-34-1.

9.5 *Water*—Distilled or deionized, 17 MΩ·cm or purer.

10. Procedure for Method B

10.1 Measure 20-g oven-dried test aliquot of compost into a 250-mL extracting bottle.

10.1.1 Prepared material shall be oven-dried at 75°C, with man-made inerts removed, and milled to attain a particle size of <1 mm. Preparation steps are described in the sample preparation section, Method 02.02-E.

10.1.2 Prepare a blank (with no compost), plus a reference sample or 0.20 to 0.50 g $CaCO_3$ powder (CAS 471-34-1). Alternatively, more or less compost can be used depending on amount of estimated limestone. Twenty g works well for materials with 1-2% lime.

10.2 Slowly add 100 mL 1 *M* HCl swirling gently. After about two minutes, cover loosely to permit release of CO_2 generated, and continue swirling occasionally for one h at ambient laboratory temperature. Allow to stand for 8 h, (e.g., overnight), then cap and mechanically shake for 1.5-2.0 h.

10.3 Filter or centrifuge after the sample suspension settles. Transfer a 10-mL aliquot of supernatant to the 100-mL Erlenmeyer flask. Add about 25 mL of deionized water. Titrate to phenolphthalein endpoint (2-3 drops of indicator) with the standardized 0.5 *M* NaOH.

11. Calculations and Corrections for Method B

11.1 Determine the calcium carbonate equivalency:

$$CaCO_3 = [M \times (B - S) \times 50] \div TS \qquad \textit{Equation 11.1}$$

where:

$CaCO_3$ = calcium carbonate equivalency, %,
M = molarity of the NAOH used,
B = volume of standard NaOH used for blank, mL,
S = volume of standard NaOH used for sample, mL, and
TS = sample aliquot size, g of oven-dried product.

04.08 METHODS SUMMARY

12. Report

12.1 *Units of Measure*—This test reports the percent of limestone equivalent to the nearest 1%.

13. Precision and Bias

13.1 Precision and bias are not determined for this test. Data are being sought to formulate a precision and bias statement.

14. Keywords

14.1 bi-carbonate, carbonate, total carbonates, limestone, pH, total inorganic carbon

Test Method: Cation Exchange Capacity. Two Methods.							**Units:** cmol kg^{-1}	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.09-A
								04.09-B

04.09 CATION EXCHANGE CAPACITY FOR COMPOST

1. Scope

1.1 This section covers the measurement of cation exchange capacity (CEC) for finished compost,

1.1.1 *Method 04.09-A CEC by Ammonium Displacement After Washing.*

1.1.2 *Method 04.09-B CEC by Direct Displacement.*

1.2 The values stated in SI units are to be regarded as the standard. The values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 03-09-A Total Solids and Moisture Content at 70±5°C.

Method 02.02-C Man Made Inert Removal and Classification.

2.2 *Other References:*

Roades, J.D. 1982. Cation Exchange Capacity. p. 149-157. *In* Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. A.L. Page ed. 2nd edition. ASA, and SSSA, Madison WI.

Soil Survey Lab Methods Manual, Soil Survey Investigations Report no. 42, Version 2.0, August 1992. Soil Conservation Service, USDA, Lincoln, NE.

US EPA Method 9080. Cation-Exchange Capacity of Soils (Ammonium acetate), Rev. 0. September 1986. *In* Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846. US EPA.

Holmgren, G.G.S., R.L. Juve, and R.C. Geschwender. 1977. A Mechanically Controlled Variable Rate Leaching Device. Soil Sci. Soc. Am. J. 41:1207-1208.

Fisher Scientific Atlanta, 3970 Johns Creek Court, Suite 500, Suwanee, GA 30024. Telephone: 770-871-4500; URL: http://www.fishersci.com.

Centurion International, Inc.; P.O. Box 82846; Lincoln, NE 68501-2846; Telephone: 800-228-4563.

3. Terminology

3.1 *anion, n*—A negatively charged ion, that migrates to an anode during electrolysis.

3.2 *cation, n*—A positively charged ion that migrates to the cathode during electrolysis.

3.3 *cation exchange capacity*, *n*—The capacity of compost, mineral and organic soils to hold exchangeable cations to counter balance the fixed negative charges in the material.

3.4 *displacement, n*—A reaction in which an atom, a radical, or a molecule replaces another in a compound.

3.5 *summation, n*—A sum or an aggregate; the act or process of adding.

3.6 *standard, n*—Serving as or conforming to a standard of measurement or value. Sample often referred to as a standard reference sample or check of known physical, chemical or biological characteristics and used to monitor analytical bias or accuracy of a physical, chemical or biological determination.

4. Summary of Test Methods

4.1 There are two basic approaches for measuring CEC, a *displacement method and a summation method.*

4.1.1 *Displacement Method*—The displacement method may be applied to all sample types including those high in soluble salts, (e.g., MSW compost). Exchange sites in the sample are saturated with either ammonium (NH_4^+) or sodium (Na^+) cations by leaching

with an acetate buffer containing either of these ions. This is similar to the first leaching in the summation method. In both techniques, CEC is expressed as cmol kg^{-1} dw.

4.1.1.1 *Method 04.09-A Ammonium Displacement After Washing*—The sample once saturated with NH_4^+, is washed with ethanol to remove all traces of the saturating cations not held by exchange sites. Following this step, the sample is leached quantitatively with a replacement solution of sodium chloride where NH_4^+ is the saturating cation. The replacement solution is analyzed with distillation and titration techniques or colorimetrically for ammonia.

4.1.1.2 *Method 04.09-B CEC by Direct Displacement*—The saturating cation is NH_4^+. After washing the sample with ethanol to remove all traces of NH_4^+ ions, the sample is quantitatively transferred to a distillation flask. Sodium chloride and a base are then added and ammonia is distilled directly from the treated sample and titrated.

4.1.2 *Summation Method (procedures not included)*—The sample is leached with an acetate salt buffer and the replaced base cations (Ca^{++}, Mg^{++}, Na^+, and K^+) are summed along with a titrated hydrogen value.

CAUTION—Cations from soluble salts cannot be determined separately from those held by exchange sites and will result in an over-estimation of the sample CEC (positive error). The summation method should not be applied to compost samples that contain excessively high soluble salts, (e.g., manure and municipal solid waste (MSW) composts). Appropriate data and further evaluation of the relative impact of, and compensation for excess soluble salts on CEC determinations is being sought to develop an interference and limitations statement.

5. Significance and Use

5.1 Cation exchange capacity (CEC) measures the capacity of compost, mineral and organic soils to hold exchangeable cations to counter balance the fixed negative charges in the material.

5.2 Sources of negative charges in compost include dissociation of acidic functional groups (e.g., OH, COOH). These charges are neutralized by various cations including K^+, Ca^{++}, Mg^{++}, and Na^+.

6. Interference and Limitations

6.1 The interference and limitations of this test are not determined. Data are being sought for use in developing an interference and limitations statement.

6.2 *Soluble Salts*—Cations from soluble salts cannot be determined separately from those held by exchange sites and will result in an over-estimation of the sample CEC (positive error). The summation method should not be applied to compost samples that contain excessively high soluble salts, (e.g., manure and municipal solid waste (MSW) composts). Appropriate data and further evaluation of the relative impact of, and compensation for excess soluble salts on CEC determinations is being sought to develop an interference and limitations statement.

7. Sample Handling

7.1 Use air-dried material, free of inerts larger in size than 4 mm, and ground to pass a 2-mm sieve, 20-mesh. Refer to Method 02.02-C Man Made Inert Removal and Classification.

Test Method: Cation Exchange Capacity. Ammonium Displacement After Washing						**Units:** cmol kg^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.09-A

04.09-A CEC BY AMMONIUM DISPLACEMENT AFTER WASHING

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTION—This test is based upon standard procedures developed for compost analysis at the Research Analytical Laboratory; Department of Soil, Water, and Climate; University of Minnesota; St. Paul, MN 55108; *by Robert Munter.*

8. Apparatus for Method A

8.1 *Mechanical Vacuum Extractor*—24-place.

8.2 *Syringes*—polypropylene, disposable, 60-mL, for sample tube, extractant reservoir, and extraction syringe.

8.3 *Plunger*—Modified. Remove rubber and cut plastic protrusion from plunger end.

8.4 *Auto Analyzer*—Kjeltec Auto 1030 Analyzer, Tecator™, or equivalent.

8.5 *Digestion Tubes*—straight neck, 250-mL, Fisher Scientific Inc., for above analyzer.

8.6 *Electronic Balance*—1 mg sensitivity.

8.7 *Sieve*—100-mesh PVC sieve.

9. Reagents and Materials for Method A

9.1 *Ammonium Acetate (1M NH_4OAc, pH 7)*—Dissolve 154.16 g of NH_4OAc in 2.0 L of double deionized water. Adjust pH to 7.0 with either ammonium hydroxide (NH_4OH) or acetic acid (CH_2COOH) if necessary.

9.2 *Boric Acid and Indicator Solution*—Dissolve 100 g of boric acid (H_3BO_3) in 10 L of double deionized water (1% solution). Add the following solutions to the above solution of boric acid:

9.2.1 Add 100 mL of methanol in which is dissolved 100 mg of bromocresol green.

9.2.2 Add 70 mL of methanol in which is dissolved 70 mg of methyl red.

9.2.3 Add 5 mL of 1*M* (4%) NaOH.

9.3 *Standardized Hydrochloric acid (HCl) 0.0200 N*—Add 80 mL of concentrated HCl to 48 L of deionized water and mix. Standardize this solution against Na_2CO_3 as a primary standard.

9.4 *Ethanol*—95%, USP.

9.5 *Sodium Chloride (NaCl)*—reagent grade.

9.6 *Filter Pulp*—Schleicher and Schuell, No. 289.

10. Procedure for Method A

10.1 *Prepare Sample Tubes*—(syringe barrel) by tightly compressing a 1-g ball of filter pulp into the bottom of the syringe barrel with the modified syringe plunger. Weigh a 1±0.002 g compost sample (air dried at 36°C and milled to pass a 100 mesh sieve) and place in the sample syringe barrel.

10.1.1 Place the sample syringe barrel in the upper disc of the extractor and connect to an inverted, tared extraction syringe with a 2.5-cm (1-in.) length of rubber tubing. Insert the plunger of the syringe in the slot of the stationary disc of the extractor.

10.1.2 Add about 10 mL of NH_4OAc to the sample allowing it to thoroughly wet for at least 20 min.

10.1.3 Put a reservoir tube on top of the sample tube and extract rapidly until NH_4OAc is at a depth of 0.5 to 1 cm above the sample.

10.1.4 Turn off the extractor and add about 45 mL NH_4OAc to the reservoir tube. Turn on the extractor and extract slowly overnight (12-16 h).

10.1.5 The next morning, turn off the extractor. Pull the plunger of the syringe down but not out of the barrel.

10.1.6 Remove the collection syringe and discard the extract.

10.1.7 Refit the emptied collection syringes to the sample tubes. Rinse sides of the sample tubes with ethanol from a wash bottle; fill sample tubes to 25-mL mark, stir, and let stand for 15 to 20 min.

10.2 *Wash Ammonium Acetate from Sample*—Return the extractor to the starting position. Attach syringe to the sample tube and rinse the sides of the sample tube with ethanol from a wash bottle. Fill the sample tube to the 20-mL mark and allow it to stand for 15 to 20 min.

10.2.1 Insert a reservoir tube into the sample tube. Rapidly extract the ethanol at a setting of 25 to 35 until the level of ethanol is 0.5 to 1 cm above the sample.

10.2.2 Turn off the extractor and add enough ethanol to reservoir to assure an excess over the capacity of the

syringe (55-60 mL). Extract at a setting of 12 (approximately 45 min).

10.2.3 After the extractor stops, turn the power switch off. Pull the plunger of the syringe down. Remove the syringe and discard the ethanol.

CAUTION—Do not pull the plunger from the syringe barrel.

10.2.4 Extract with ethanol a second time, but omit stirring the samples. This flushes the excess NH_4^+ from the soil so that the only NH_4^+ remaining is that bound to the cation exchange sites. Discard the ethanol.

10.3 Extract the sample with acidified 10% KCl.

10.3.1 To remove the NH_4^+ bound to the sample, extract the sample with acidified 10% KCl in place of the NH_4OAc in steps 10.1.2-10.1.5, above. This large flush of K^+ will serve to replace the bound NH_4^+.

10.3.2 Weigh the syringes containing KCl extract (±0.01 g), mix thoroughly and set aside the extract for determination of NH_4^+ (Method 04.02-C).

10.3.3 Distill the KCl extract on the Kjeltec Auto 1030 Analyzer to measure the NH_4^+ levels. (See the Kjeltec instruction manual for specific operating instructions.)

10.4 *Blanks and Reference Samples:*

10.4.1 Include a minimum of two blanks per batch of forty samples.

10.4.2 Include a known laboratory reference sample that is routinely analyzed per batch of 20 samples.

11. Calculation for Method A

11.1 *Calculate Cation Exchange Capacity (cmol kg^{-1} dw):*

$$CEC = [T \times N \times V \times 100 \times A \div O] \div F \qquad \textit{Equation 11.1}$$

where:

CEC = cation exchange capacity, cmol kg^{-1},
T = titer (mL sample titer minus blank titer),
N = normality of standard acid,
V = total volume (mL) of filtrate collected,
A÷O = (air dry weight, 36°C) ÷ (oven dry weight, 70±5°C), and
F = Aliquot (mL) of filtrate distilled.

Test Method: Cation Exchange Capacity: Direct Displacement						**Units:** cmol kg^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								04.09-B

04.09-B CEC BY DIRECT DISPLACEMENT

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTION—This method was prepared by Robert Munter

12. Apparatus for Method B

12.1 *Mechanical Vacuum Extractor*—24-place.

12.2 *Syringes*—polypropylene, disposable, 60-mL, for sample tube, extractant reservoir, and extraction syringe.

12.3 *Plunger*—Modified. Remove rubber and cut plastic protrusion from plunger end.

12.4 *Auto Analyzer*—Kjeltec Auto 1030 Analyzer, Tecator™.

12.5 *Digestion Tubes*—straight neck, 250-mL, Fisher Scientific Inc., for above analyzer.

12.6 *Analytical Balance*—1-mg sensitivity.

13. Reagents for Method B

13.1 *Ammonium Acetate (1M NH_4OAc, pH 7)*—Dissolve 154.16 g of NH_4OAc in 2.0 L of double deionized water. Adjust pH to 7.0 with either ammonium hydroxide (NH_4OH) or acetic acid (CH_2COOH) if necessary.

13.2 *Boric Acid and Indicator Solution*—Dissolve 100 g of boric acid (H_3BO_3) in 10 L of double deionized water (1% solution). Add the following solutions to the above solution of boric acid:

13.2.1 Add 100 mL of methanol in which is dissolved 100 mg of bromocresol green.

13.2.2 Add 70 mL of methanol in which is dissolved 70 mg of methyl red.

13.2.3 Add 5 mL of 1*M* (4%) NaOH.

13.3 *Standardized Hydrochloric Acid (0.0200 N, HCl)*—Add 80 mL of concentrated HCl to 48 L of deionized water and mix. Standardize this solution against Na_2CO_3 as a primary standard.

13.4 *Ethanol*—95%, USP.

13.5 *Sodium Chloride (NaCl)*—reagent grade.

13.6 *Filter Pulp*—Schleicher and Schuell, no. 289.

14. Procedure for Method B

14.1 Prepare sample tubes:

14.1.1 *Syringe Barrel*—tightly compress a 1-g ball of filter pulp into the bottom of the syringe barrel with the modified syringe plunger. Weigh a 1±0.002 g compost sample (air dried at 36°C and milled to pass a 100 mesh sieve) and place in the sample syringe barrel.

14.1.2 Place the sample syringe barrel in the upper disc of the extractor and connect to an inverted, tared extraction syringe with a 2.5 cm (1-in.) length of rubber tubing. Insert the plunger of the syringe in the slot of the stationary disc of the extractor.

14.1.3 Add about 10 mL of NH_4OAc to the sample allowing it to thoroughly wet for at least 20 min.

14.1.4 Put a reservoir tube on top of the sample tube and extract rapidly until NH_4OAc is at a depth of 0.5 to 1 cm above the sample.

14.1.5 Turn off the extractor and add about 45 mL NH_4OAc to the reservoir tube. Turn on the extractor and extract slowly overnight (12-16 h).

14.1.6 The next morning, turn off the extractor. Pull the plunger of the syringe down but not out of the barrel.

14.1.7 Remove the collection syringe and discard the extract.

14.1.8 Refit the emptied collection syringes to the sample tubes. Rinse sides of the sample tubes with ethanol from a wash bottle; fill sample tubes to 25-mL mark, stir, and let stand for 15 to 20 min.

14.2 Remove the sample tube from the extract and transfer the sample with filter pulp to a 250-mL digestion tube. Add 7 g of NaCl to the digestion tube. Use a gentle flow of compressed air to blow the filter pulp and sample out of the syringe. Wash the tube with deionized water and use a rubber policeman to complete the transfer. The amount of distilled water that is added depends on the amount that is required to complete the transfer of tube contents.

14.3 Perform the same transfer and addition of reagents for blanks as for samples.

14.4 Spray silicone antifoam agent (or 2 drops of octyl alcohol) into the digestion tubes for each of the samples and reagent blanks.

14.5 Connect the tube to the distillation unit. Close the safety door. Distillation and titration are performed automatically. Record the titer in mL of titrant and calculate cmol ammonia in the equivalent of 100 g air dry sample. Correct data to 70±5°C basis by determining oven dry moisture on a separate aliquot of air-dry sample.

14.6 *Blanks and Reference Samples:*

14.6.1 Include a minimum of two blanks per batch of forty samples.

14.6.2 Include a known laboratory reference sample that is routinely analyzed per batch of 20 samples.

15. Calculations for Method B

15.1 *Calculate Cation Exchange Capacity (cmol kg^{-1} dw):*

CEC = [T × *N* × V × 100 × A ÷ O] ÷ F *Equation 15.1*

where:

CEC = cation exchange capacity,
T = titer (mL sample titer minus blank titer),
N = normality of standard acid,
V = total volume (mL) of filtrate collected,
A÷O = [Air Dry Weight, 36°C] ÷ [Oven Dry Weight, 70±5°C], and
F = Aliquot (mL) of filtrate distilled.

04.09 METHODS SUMMARY

16. Report

16.1 *Methods 04.09-A and 04.09-B*—Report determination at ±0.1 cmol kg^{-1}.

16.1.1 *Minimum Detectable Concentration*—Detection limit of 0.5 cmol kg^{-1}

17. Precision and Bias

17.1 *Method 04.09-A*—The precision of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

17.1.1 The reference material was oven-dried at 70°C, passed through a 9.5-mm sieve, and milled to fine powder with a Stein Mill and stored in a sealed container at room temperature (~27°C).

Table 04.09-A1 Precision for Standard Compost Reference Material (cmol kg^{-1}). Variability is expressed as percent coefficient of deviation.

Mean	*Std Dev*	*% CV*	*N*
36.5	1.2	3.2	9

Table 04.09-A2 Precision (variability) of CEC determinations (cmol kg^{-1}) for field composited samples from each of two sampling periods (during 1993) at three municipal solid waste (MSW) composting different facilities.

Mean	*Std Dev*	*%CV*	*N*
21.8	1.0	5	10
29.1	0.6	2	10
28.9	0.5	2	10
15.1	2.0	13	10
26.4	2.5	9	10
31.9	1.1	3	10

17.2 *Method 04.09-B CEC by Direct Displacement*—The precision and bias of this test are not determined. Data are being sought for use in developing a precision and bias statement.

18. Keywords

18.1 cation exchange capacity; CEC; displacement

Test Method: Electrical Conductivity for Compost. One Method							**Units:** dS m^{-1}	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.10-A	04.10-A							04.10-A

04.10 ELECTRICAL CONDUCTIVITY FOR COMPOST

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

NOTE 1—1 Mhos ≡ 1 Siemen's unit ≡ 1 Ω^{-1}

1. Scope

1.1 This section covers the determination of electrical conductivity of compost.

1.1.1 *Method 04.10-A 1:5 Slurry Method, Mass Basis.*

NOTE 2—The 1:5 Slurry method is included in TMECC while the Saturated Paste Extract method (ECe) was removed with peer agreement through the TMECC peer-review process in the interest of diminishing variations in reported EC results for compost samples. The 1:5 Slurry method is more conservative, analytically sound and less prone to systematic error. It also includes sample preparation steps that account for variations in moisture content among compost samples. The 1:5 Slurry method is valid for use on compost samples that are not amended with inorganic fertilizers, (e.g., ammonium sulfate, etc.) which significantly increase measured EC values.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 03.09 Total Solids and Moisture.

Method 04.11 Electrometric pH Determinations for Compost.

Method 05.02-E Agricultural Index (AgIndex).

2.2 *Other References*:

NCR Pub. No. 221 (Revised), Recommended chemical soil test procedures, Missouri agricultural experiment station SB 1001, January 1998.

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US Salinity Laboratory Staff. 1954. Diagnosis and improvement of saline and alkali soils. USDA Handbook No. 60. p 90. U. S. Govt. Print. Office. Washington, DC.

Rhoades, J.D. 1996. Salinity: Electrical conductivity and total dissolved solids. p. 417-435. *In* J. M. Bartels et al. (ed.) Methods of Soil Analysis: Part 3. Chemical Methods 3rd. ed. ASA and SSSA, Madison, WI. Book series no. 5.

Peters, J. R. 1963. The nature and management of saline soils. Manitoba Dept. of Agric. and Conservation. Publ. #360.

3. Terminology

3.1 *salt, n*—A chemical compound formed by replacing all or part of the hydrogen ions of an acid with metal ions or electropositive radicals.

3.2 *standard, n*—Serving as or conforming to a standard of measurement or value. Sample often referred to a standard reference sample or check of known physical, chemical or biological characteristics used to monitor analytical bias or accuracy of a physical, chemical or biological determination.

4. Summary of Test Methods

4.1 *Method 04.10-A 1:5 Slurry Method, Mass Basis*—A compost sample at as-received moisture is blended with water at a ratio of 1:5, dw/v equivalent basis. The sample is shaken for 20 min at room temperature to allow the salts to solubilize in the water. Electrical conductivity is measured in the 1:5 sample slurry. An optional extraction step is provided for situations where a conductivity measure is required for the sample extract solution.

5. Significance and Use

5.1 Electrical conductivity is a measure of the soluble salt content in compost, where soluble salts refers to the concentration of soluble ions in a solution. Conductivity varies with both the number and type of ions contained in the solution, and can indicate if a compost has phytotoxic potential when used as a soil amendment.

5.2 Pure water is a very poor conductor of electric current, whereas water containing the dissolved ions ordinarily found in compost and soil conducts current approximately in proportion to the amount of cations and anions present in solution. Therefore, electrical conductivity measurements of a compost extract provides an indication of the total ion concentration in the matrix.

5.3 Soluble salts in compost may limit its ultimate end use. Each user group, (e.g., vegetable growers, nursery industry, etc.), has its own set of salinity standards for growing specific plants or crops.

5.4 High salt determinations for a given compost or feedstock may be associated with high plant nutrient content, but can damage plants—especially seedlings by burning roots and preventing or delaying germination.

5.5 Excessively high electrical conductivity (ion content) decreases plant-available soil water and plant nutrient uptake. Conversely, very low salt content may indicate low fertility levels, especially of bases such as potassium, calcium or magnesium. Refer to Method 05.02-F to manage the nutrient to salt ratio (AgIndex) of feedstocks and grading finished composts for nutrients.

6. Interference and Limitations

6.1 *Inorganic fertilizers*—The 1:5 Slurry method is valid for use on compost samples that are not amended with inorganic fertilizers, (e.g., ammonium sulfate, etc.). Inorganic fertilizers will significantly increase measured electrical conductivity values.

6.2 *Probe maintenance*—composts are often high in organic acids. These acids can accumulate on the surface of the glass electrodes forming a film and diminishing the flow of current. See manufacturer's instructions for cleaning and/or recoating the electrodes.

6.3 Use deionized water with minimum resistivity of 17 $M\Omega \cdot cm^{-1}$. Do not use tap water because it often carries significant concentrations of minerals such as Ca, Mg, K, Cl and other salts that will distort the electrical conductivity measurement.

6.4 The electrical conductivity of an aqueous salt solution increases with increasing temperature (approximately 2% per °C). The standard temperature for reporting electrical conductivity measurements is 25°C. If the conductivity/resistivity meter used does not compensate for differing temperature, a correction factor must be applied.

6.5 *Optional Extraction Step*—Centrifugation does not always provide complete removal of solution extract as does the vacuum extraction approach. For this reason, vacuum extraction is called for in the optional extraction steps.

7. Sample Handling

7.1 Use as-received, moist material with a maximum aggregate size of 9.5 mm, i.e., material that passes through a 9.5-mm sieve.

7.1.1 *Method 04.10-A*—This method requires approximately 30 cm^3 or less of material for determining electrical conductivity of a sample. The prepared slurry or extract for this method can also be used for *Method 04.11-A Electrometric pH Determinations.*

Test Method: Electrical Conductivity for Compost. 1:5 Slurry Method, Mass Basis							**Units:** dS m^{-1}	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.10-A	04.10-A							04.10-A

04.10-A 1:5 SLURRY METHOD, MASS BASIS

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Apparatus for Method A

8.1 *Conductivity/Resistivity Meter*—soluble salt bridge, (e.g., Industrial Instrument Inc., model RC-16B2 or equivalent).

8.2 *Stirring Rod*—approximately 15-cm length, glass.

8.3 *Conductivity Cell*—1-cm, apparatus-specific.

8.4 *Sample Flasks*—500-mL, plastic or glass Erlenmeyer flasks, with screw-cap lid or cover.

8.5 *Sample Beakers*—100-mL, plastic or glass.

8.6 *Reciprocating Shaker*—capable of shaking a sample flask at the rate of 180 reciprocations or excursions per min.

8.7 Centrifuge Extraction Apparatus (optional step)—200-mL centrifuge tubes, capable of 8000 *g*.

9. Reagents and Materials for Method A

9.1 *Water*—ammonia-free, carbonate-free, deionized, minimum resistivity of 17 $M\Omega \cdot cm^{-1}$.

9.2 *Calibration Standard*—Dissolve 0.7456 g KCl (previously dried at 110°C for 2 h) deionized water and dilute to 1.0 L. At 25°C±0.1°C a 0.010 *N* KCl solution will have an EC of 1.412 dS m^{-1} (mmhos cm^{-1}). For a 0.100 *N* KCl solution (7.456 g KCl diluted to 1.0 L) will have an EC of 12.900 dS m^{-1}. Standard EC calibration solutions are listed in Table 04.10-A1 and can be purchased from a scientific supply vendor.

9.3 *Filter paper (optional step)*—medium flow, Whatman No. 1 or equivalent.

10. Procedure for Method A

10.1 *Calibration Check*—Determine conductivity of calibration solutions. Refer to Table 04.10-A1.

Table 04.10-A1 Conductivity of KCl solutions at 25°C

Normal Concentration	Conductivity (dS m^{-1})
0.001	0.147
0.010	1.413
0.020	2.767
0.050	6.668
0.10	12.90
0.20	24.82
0.50	58.64

ADAPTED FROM—Rhoades, 1996

10.2 *Duplicate Samples*—Within each batch of twelve samples duplicate at least one sample to monitor precision.

10.3 *Compost Aliquot Moisture*—Determine the total solids ratio on a parallel sample aliquot.

10.3.1 Measure and record the as-received tare weight of the aliquot. Oven dry the aliquot in a microwave oven with high temperature setting for approximately 5 min, or until sample weight-change diminishes to nill. Calculate the total solids ratio by dividing the microwave oven dry weight by the as-received moist weight.

CAUTION—Metal fragments, i.e., inert contaminants in the compost aliquot, may cause the sample to ignite inside of the microwave oven.

10.3.2 If no microwave oven is available, follow the protocols to determine total solids as described in Method 03.09 Total Solids and Moisture, the procedure required for reporting sample moisture content. This choice will require that Method 04.10-A is performed after the total solids and moisture determination is completed.

10.4 *Prepare Samples:*

10.4.1 Weigh 40.0 g dry-weight equivalent of as-received moist compost (Equation 10.4.1.1) into the sample container, (e.g., 250-mL screw-cap flask).

10.4.1.1 Determine the dry-weight equivalent aliquot size.

$$A = B \div [C \times 0.01] \qquad \textit{Equation 10.4.1.1}$$

where:

A = mass of as-received moist compost aliquot, g
B = dry-weight equivalent of sample, 40.0 g,
C = sample total solids content, % wet weight basis, and
0.01 = factor to convert from percentage to fraction, unitless.

10.4.2 Bring the liquid fraction of the 1:5 solids:liquid slurry to an equivalent of 200 mL by adding deionized water to the as-received moist compost aliquot (refer to Equation 10.4.2.1). This step is based upon an assumption that 1 mL is equivalent to 1 g of the as-received compost liquid fraction, and that 1 mL of water is equivalent to 1 g of water.

10.4.2.1 Determine the required volume of extractant.

$$A = B - [C - 40] \qquad \textit{Equation 10.4.2.1}$$

where:

A = volume of extractant required, mL
B = target 1:5 slurry liquid fraction, 200 mL
C = mass of as-received compost aliquot, g, and
40 = total solids fraction of the as-received compost aliquot, g.

10.4.3 Place the 250-mL flasks with the 1:5 slurry on a shaker for 20 min at 180 reciprocations or excursions per minute.

10.4.4 Maintain slurry at ambient laboratory temperature, (e.g., 20°C to 23°C).

10.5 *Optional Extraction Step*—Extract the 1:5 solids:liquid slurry liquid fraction. Determine conductance on extact rather than on the slurry as described below. Report the inclusion of this step when reporting analytical results

10.5.1 Transfer the slurry to a 200-mL centrifuge tube. Centrifuge at 8000 *g* for fifteen min to separate solid and liquid fractions, or

10.6 *Electrical Conductance*—Determine the electrical conductance of the 1:5 compost/water slurry with a conductivity/resistivity meter.

NOTE 1A—If the conductivity meter requires the use of a 1-cm conductivity cell, incorporate the optional extraction step (14.4) and proceed with the extract solution rather than the slurry as described below.

10.6.1 Standardize the conductivity meter using the standard KCl solution following manufacturers instructions.

10.6.2 Measure the temperature of the slurry. Set the temperature compensation dial on the conductivity meter to the temperature of the slurry.

10.6.3 Insert the conductivity electrodes into the slurry and swirl gently. Allow the instrument/sample to stabilize. Read and record the conductivity of the slurry (dS m^{-1} = mMhos cm^{-1}).

NOTE 2A—If the conductivity meter does not have a temperature compensator, follow the temperature correction formula provided in the appendix of this section, 04.10 Appendix. Temperature Correction.

11. Calculations and Corrections for Method A

11.1 If temperature compensation is not an option in the conductivity meter, correct the reading to 25°C as specified in 04.10 APPENDIX—Temperature Correction.

04.10 METHODS SUMMARY

12. Report

12.1 Report method selected for electrical conductivity determination, sample pH, as-received moisture content, material type, (e.g., compost, feedstock, etc.), and source material, (e.g., MSW, biosolids, yard waste, etc.).

12.1.1 *Optional Extraction Step*—Report use of this step and all other protocol modifications that deviate from the write-up.

12.1.2 *Minimum Detectable Concentration*—±0.1 dS m^{-1} (mMhos cm^{-1}).

13. Precision and Bias

13.1 *Method 04.10-A 1:5 Slurry Method, Mass Basis*—The precision and bias of this test are not determined. Data are being sought for use in developing a precision and bias statement.

14. Keywords

14.1 soluble salts; electrical conductivity; dilution; slurry; extract; saturation; 1:5

APPENDIX TO 04.10—TEMPERATURE CORRECTION

15. Temperature Correction for Methods 04.10-A.

15.1 *Temperature Correction Coefficient*—Measure electrical conductivity of the KCl calibration standard at laboratory temperature. Divide the 25°C standard electrical conductivity value by the measured value.

$$A = B \div C \qquad \textit{Equation 15.1}$$

where:

A = temperature correction coefficient, unitless,

B = conductivity of KCl calibration standard at 25°C, 1.41 dS m^{-1}, etc. (refer to Table 04.100-A1), and

C = electrical conductivity of KCl calibration standard at laboratory temperature, dS m^{-1}, (mMhos cm^{-1}).

15.2 Multiply the reading from each sample by the temperature correction coefficient to correctly report readings on a 25°C basis.

$$D = E \times A \qquad \textit{Equation 15.2}$$

where:

D = corrected reading for sample on a 25°C basis, dS m^{-1} (mMhos cm^{-1}),

E = reading for sample at laboratory temperature, dS m^{-1} (mMhos cm^{-1}),

A = temperature correction coefficient, unitless.

Test Method: Electrometric pH Determinations for Compost. One Method						**Units:** pH		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.11-A	04.11-A	04.11-A	04.11-A		04.11-A		04.11-A

04.11 ELECTROMETRIC pH DETERMINATIONS FOR COMPOST

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the determination of pH of compost and compost feedstocks.

1.1.1 *Method 04.11-A 1:5 Slurry pH.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 04.10 Electrical Conductivity for Compost.

2.2 *Other References:*

Eckert, D.J. 1988. Recommended pH and lime requirement tests. *In* Recommended Chemical Soil Test Procedures for the North Central Region. North Dakota Agric. Exp. Stn. Bull. 499. Fargo, N.D.

NCR (North Central Regional) Method 14. 1988. pp. 34-37. *In* Recommended Test Procedure for Greenhouse Growth Media NCR Pub No. 221 (Rev), *Recommended Chemical Soil Test Procedures*, Bulletin Number 499 (Rev), October 1988.

Soils and Soil Fertility. 5th Edition. F. R. Troeh and L. M. Thompson, ed. Collage of Agriculture. Iowa State University. Oxford University Press. 1993.

US EPA Method 9045, Soil pH. *In* Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, US EPA SW-846, 3rd Edition, November 1992.

Warncke, D. 1998. Greenhouse root media. pp. 61-64. *In* Recommended chemical soil test procedures for the North Central Region. North Central Regional Research Publication No. 221 (Revised) Missouri Agricultural Experiment Station SB 1001.

Watson, M.E. and J.R. Brown. 1998. pH and lime requirement. pp. 13-16. *In* Recommended chemical soil test procedures for the North Central Region. North Central Regional Research Publication No. 221 (Revised) Missouri Agricultural Experiment Station SB 1001.

3. Terminology

3.1 *pH, n*—A measure of the acidity or alkalinity of a solution, numerically equal to 7.0 for neutral solutions, increasing with increasing alkalinity and decreasing with increasing acidity. The pH scale commonly in use ranges from 0 to 14, measures the negative log of hydrogen ion concentration (activity).

4. Summary of Test Methods

4.1 *Method 04.11-A 1:5 Slurry pH*—A slurry of compost and deionized water is blended at a ratio of 1:5, w/w or v/v basis. The sample is shaken for 20 min at room temperature to allow the salts to solubilize in the DI water. The pH is measured with an electrometric pH meter directly in the compost/water slurry or in the extracted solution. An optional extraction step is provided for situations where a pH measure is required for the sample extract solution. The measurement of pH is expressed as the negative log of the hydrogen ion activity. Activity and concentration are similar if the salt concentration is low.

5. Significance and Use

5.1 pH influences many factors in compost, including the availability of nutrients and toxic substances, and activities and nature of microbial populations. The pH affects the composting process by affecting the microbial population and by controlling availability of nutrients to microbes. The optimum pH lies between 6.0 and 7.5 for most bacteria, while the optimum pH for fungi and actinomycete activity is between 5.5 and 8.0. A pH below and higher than a specified optimum will reduce microbial activity and curtail or arrest biological processes.

5.2 In addition, pH is both an indicator of compost quality and a useful tool for determining its potential application. The pH of a compost will determine if the user needs to amend the compost to adjust the pH for a

particular application. It indicates compost stability, and phytotoxicity and is an indicator of metal and nutrient mobility and availability in compost.

5.3 High soil/compost pH (>7.0) decreases the transfer of cadmium and other metals into the food chain, and the potential for metal phytotoxicity.

5.4 The relationship of *soil* pH and plant nutrient availability is illustrated in *Fig 04.11-1. Relative availability of plant required nutrient at varying pH levels.* The thickness of each bar varies with pH, bar thickness represents relative availability of the nutrient listed at the left hand margin of the figure. Small text at opposite ends of each bar indicates a dominant factor that often causes decreased nutrient availability. Nitrogen is readily available from approximately pH 6.0 to pH 8.0, but diminishes at lower and higher pH's as microbial activity declines. Phosphorus availability diminishes at lower pH's (below 5.0) as it readily binds with aluminum and iron, and diminishes at higher pH's (approximately 8.0), as it readily binds calcium. The optimal pH ranges for phosphorus and potassium are similar. Unlike phosphorus, potassium solubility increases with decreasing pH, but it can be less available at low pH because of leaching. Calcium, magnesium, copper and boron solubility increase with decreasing pH and also become less available through leaching. These metals become insoluble by forming carbonates and oxides at high pH's. Molybdenum is different because it is insoluble and unavailable at low pH's.

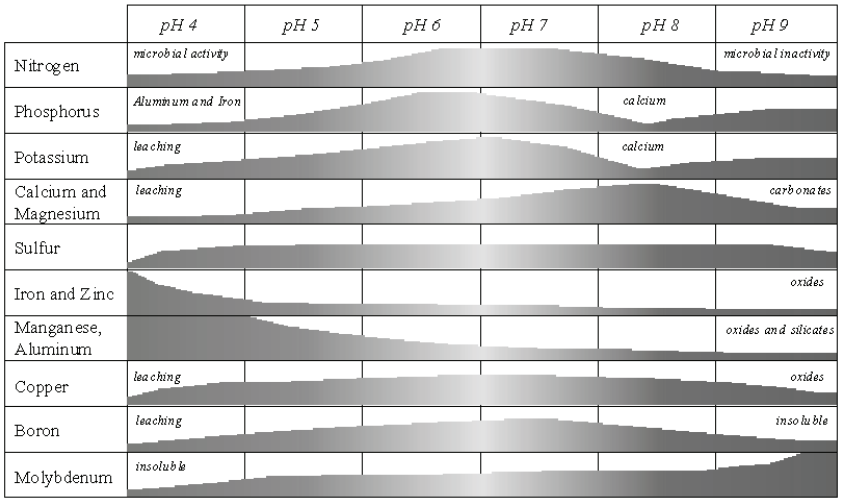

Fig 04.11-1 Relative availability of plant required nutrients in **soil** at varying pH levels.

ADAPTED FROM—Soils and Soil Fertility, 5th Ed. 1993, by F. R. Troeh and L. M. Thompson.

6. Interference and Limitations

6.1 *Protect Electrodes*—When swirling samples with the electrodes immersed, be careful not to force the electrodes against the bottom of the cup or beaker because even a small scratch on the glass electrode will cause damage and reduce the accuracy of measurements.

6.2 *Measurement Accuracy*—If the reference sample reading deviates from ± 0.1 pH units, recalibrate with both buffer solutions. Check the meter calibration every 10-12 samples.

6.3 *Soluble Salts*—A small error in the pH value may result when electrical conductivity is high (soluble salt content). Appropriate data and further evaluation of the relative impact of, and compensation for excess soluble salts on pH determinations is being sought to develop an interference and limitations statement.

7. Sample Handling

7.1 Use as-received moist compost with aggregate sizes of 9.5 mm and less for this test (e.g., pass the sample material through a 9.5-mm sieve, refer to Method 02.02-B, paragraph 13.3). Sample material can be refrigerated at 4°C for up to two d.

Test Method: Electrometric pH Determinations for Compost. 1:5 Slurry pH						**Units:** pH		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	04.11-A	04.11-A	04.11-A	04.11-A		04.11-A		04.11-A

04.11-A 1:5 SLURRY pH

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Apparatus for Method A

8.1 *pH Meter*—bench top pH/ISE Meter, (e.g., Model 720A ORION No. 0720AO or equivalent).

8.2 *Glass Electrode*—hydrogen electrode.

8.3 *Reference Electrode*—silver-silver chloride or Hg calomel internal.

8.4 *Cups*—glass or plastic disposable, 37-mL (1.5 oz).

8.5 *Stirring Rod*—plastic.

8.6 *Sample Flasks*—250-mL, hard plastic or glass flasks, with screw-cap lid or cover.

8.7 *Shaker*—capable of shaking a sample flask at the rate of 180 reciprocations or excursions per min.

8.8 *Centrifuge Extraction Apparatus (optional step)*—200-mL centrifuge tubes, capable of 8000 *g*.

9. Reagents and Materials for Method A

9.1 *Reference Solutions*—commercial buffer, pH 7.0 and 10.0.

9.2 *Deionized Water*—minimum resistivity of 17 MΩ·cm, minimum standard.

10. Procedure for Method A

10.1 *pH Meter Calibration*—Calibrate the pH meter following manufacturer's instructions to the potential of the electrode pair with the pH 7.0 and 10.0 standard commercial buffer solutions with an accuracy of ±0.05 units.

10.1.1 Recalibrate if necessary. Rinse the electrode between readings of the buffer solutions. After rinsing, gently blot the tip of the electrode by touching once with a soft paper towel or tissue.

10.2 *Compost Aliquot Moisture*—Determine the total solids ratio on a parallel sample aliquot.

10.2.1 Measure and record the as-received tare weight of the aliquot. Oven dry the aliquot in a microwave oven with high temperature setting for approximately 5 min, or until sample weight-change diminishes to nill. Calculate the total solids ratio by dividing the microwave oven dry weight by the as-received moist weight.

CAUTION—Metal fragments, i.e., inert contaminants in the compost aliquot, may cause the sample to ignite inside of the microwave oven.

10.2.2 If no microwave oven is available, follow the protocols to determine total solids as described in Method 03.09 Total Solids and Moisture, the procedure required for reporting sample moisture content. This choice will require that Method 04.10-A is performed after the total solids and moisture determination is completed.

10.3 *Prepare Samples:*

10.3.1 Weigh 40.0 g dry-weight equivalent of as-received moist compost (Equation 10.3.1.1) into the sample container, (e.g., 250-mL screw-cap Erlenmeyer flask).

10.3.1.1 Determine the dry-weight equivalent aliquot size.

$$A = B \div [C \times 0.01] \qquad \textit{Equation 10.3.1.1}$$

where:

A = mass of as-received moist compost aliquot, g
B = dry-weight equivalent of sample, 40.0 g,
C = sample total solids content, % wet weight basis, and
0.01 = factor to convert from percentage to fraction, unitless.

10.3.2 Bring the liquid fraction of the 1:5 solids:liquid slurry to an equivalent of 200 mL by adding deionized water to the as-received moist compost aliquot (refer to Equation 10.3.2.1). This step is based on the assumption that 1 mL is equivalent to 1 g of the as-received compost liquid fraction, and that 1 mL of water is equivalent to 1 g of water.

10.3.2.1 Determine the required volume of extractant.

$$A = B - [C - 40] \qquad \textit{Equation 10.3.2.1}$$

where:

A = volume of deionized water required, mL
B = target 1:5 slurry liquid fraction, 200 mL
C = mass of as-received compost aliquot, g, and
40 = total solids fraction of the as-received compost aliquot, g.

10.3.3 Place the 250-mL flasks with the 1:5 slurry on a shaker for 20 min at 180 reciprocations or excursions per minute.

10.3.4 Maintain slurry at ambient laboratory temperature, (e.g., 20-23°C).

10.4 *Determination of pH*—Mix the sample slurry [or extract] with the plastic rod. Immerse the pH electrodes into the slurry [or extract]. After the meter is stabilized, carefully swirl the sample cup without lifting it, and look for a change in the meter reading. If a change occurs, swirl again until a stable reading is obtained. Record to the nearest 0.1 pH unit.

10.4.1 Rinse electrodes between sample readings with deionized water. Check electrode calibration with pH 7.0 buffer every 10 samples.

10.5 Verify the accuracy of the electrodes by including a reference sample.

10.5.1 Replicate this standard sample at approximately 8 to 24 sample intervals to ensure measurements remain accurate.

10.5.2 *Duplicate Samples*—Within each batch of samples duplicate at least one sample per 10 to monitor precision.

04.11 METHODS SUMMARY

11. Interpretation of Results

11.1 A low pH for compost of approximately 3.0 indicates that the compost is anaerobic. At low pH, H^+ ions, sulfide, aluminum and manganese ions can reach toxic levels.

12. Report

12.1 The measurement of pH is expressed as the negative log of the hydrogen ion activity of a thin aqueous slurry of compost and deionized water. Activity and concentration are similar if the salt concentration is low.

12.2 *Minimum detectable concentration*—pH meters can be accurately calibrated to ±0.05 units. The pH should be reported to the nearest 0.1 unit.

12.3 Report the electrical conductivity method preparation used, i.e., Method 04.10-A or 04.10-B, with or without the extraction step, the as-received moisture content, compost material type (e.g., compost, feedstock, etc.), and source material (e.g., MSW, biosolids, yard waste, etc.).

12.3.1 *Optional Extraction Step*—Report use of the extraction step and all other protocol modifications that deviate from the write-up.

12.3.2 *Minimum Detectable Concentration*—±0.1 mMhos cm^{-1}.

13. Precision and Bias

13.1 An electrometric pH meter that is calibrated with standard buffer solutions should be precise to ±0.05 units. The variability within a mixed sample representing the compost in question is generally less than ±0.1 units.

13.2 *Method 04.11-A 1:5 Slurry pH*—The precision and bias of this test are not yet determined. Data are being sought for use in developing a precision and bias statement.

14. Keywords

14.1 pH; electrometric pH, 1:5 solids:liquid slurry, extract, saturation

Test Method: Digestion Techniques. Five Methods							**Units:** *NA*	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.12-A	04.12-A						04.12-A	04.12-A
04.12-B	04.12-B						04.12-B	04.12-B
04.12-C	04.12-C							04.12-C
04.12-D	04.12-D	04.12-D		04.12-D				04.12-D
04.12-E	04.12-E							04.12-E

04.12 DIGESTION TECHNIQUES

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the digestion procedures for compost and compost feedstocks.

1.1.1 *Method 04.12-A Microwave-Assisted Nitric Acid Digestion of Compost, US EPA 3051 Modified for Compost.*

1.1.2 *Method 04.12-B Nitric Acid Digestion of Compost and Soils.*

1.1.3 *Method 04.12-C Dry Ash Sample Digestion for Plant Nutrients.*

1.1.4 *Method 04.12-D Water-Soluble Elements.*

1.1.5 *Method 04.12-E Aqua Regia Procedure.*

NOTE 1—*Nitric-Perchloric Acid Digestion*—This highly caustic digest is appropriate for totals determinations on many matrices, but can be highly problematic with complex materials such as compost. The degestion protocol for nitric-perchloric acid is not included in TMECC. Complete digest as required for regulatory reporting may be accomplished with less caustic reagents and should be evaluated before routine use of the nitric acid-perchloric acid digest is adopted.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC Methods:*

Method 02.02-B Sample Sieving for Aggregate Size Classification

Method 02.02-C Man-Made Inert Removal and Classification

Method 02.02-E Milling and Grinding Samples, Munter Method

2.2 *Other Sources:*

AOAC Method 985.01, Metals and Other Elements in Plants. p. 42. In Official Methods of Analysis, Agricultural Chemicals, Contaminants, Drugs. Vol. 1. 15th Edition. 1990. K. Helrich, ed. AOAC, Inc., Suite 400, 2200 Wilson Blvd., Arlington, VA 22201.

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3. Terminology

3.1 *digest, v*—To soften or disintegrate by means of chemical action, heat, or moisture. To undergo exposure to heat, liquids, or chemical agents including acid to bring elements of interest, such as metals, into solution.

3.2 *hydrochloric acid, n*—A clear, colorless, fuming, poisonous, highly acidic aqueous solution of hydrogen chloride, HCl, used as a chemical intermediate and in petroleum production, ore reduction, food processing, pickling, and metal cleaning. It occurs naturally in the stomach in dilute form.

3.3 *nitric acid, n*—A transparent, colorless to yellowish, fuming corrosive liquid, HNO_3, a highly reactive oxidizing agent used in the production of fertilizers, explosives, and rocket fuels and in a wide variety of industrial metallurgical processes. Also known as aqua fortis.

3.4 *perchloric acid, n*—A clear, colorless liquid, $HClO_4$, explosively unstable under some conditions, that is a powerful oxidant used as a catalyst and in explosives.

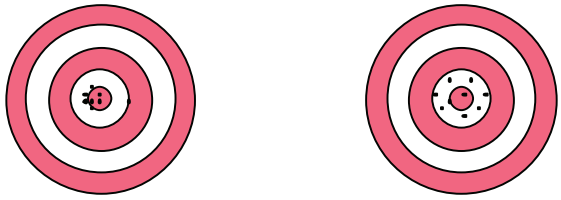
3.5 *standard, n*—Serving as or conforming to a standard of measurement or value. Sample often referred to a standard reference sample or check of known physical, chemical or biological characteristics used to monitor analytical bias or accuracy of a physical, chemical or biological determination.

3.6 *water soluble, n*—Elements detected in a water-based extraction solution after passing through a 0.45µm filter.

4. Summary of Test Methods

4.1 When selecting the digestion method, consider the minimum acceptable level of precision and accuracy across sample replicates. If moderately large variations are acceptable, consider an open system technique where sample preparation does not require milling sample material to a fine powder. When high precision across replicate sample runs is desired, choose a closed system digest and a small aliquot of a well-blended, large, milled sample.

4.2 *Open System Digests*—the following digestion techniques allow volatile components to leave a sample during the digestion or ashing process.



US EPA Method 3051 US EPA Method 3050A

Fig 04.12-1 Relative precision provided by a closed vessel digest method (US EPA 3051) where sample preparation requires milling sample material to a fine powder versus an open vessel digest method (US EPA 3050B) where milling is optional and coarse sample material may be digested.

ADAPTED FROM—Test Methods for Evaluating Solid Waste. Volume 1A. Chapter 3, Metallic Analytes. November 1986. US EPA SW-846. 3rd ed.

4.2.1 Nitric Acid - Perchloric Acid Digestion (refer to SSSA Book Series 5. Methods of Soil Analysis, Part 3. Chemical Analysis for protocols).

4.2.2 Method 04.12-B—Nitric Acid Digestion of Compost and Soils.

Table 04.12-1 Acids used in conjunction with HNO_3 for sample preparation.

Acid	*Recom-mended for...,*	*May be helpful for...,*	*Not Recom-mended for...,*
HCl	-	Sb, Ru, Sn, Fe	Th, Pb
H_2SO_4	Ti	-	Ag, Pb, Ba
$HClO_4$	-	Organic materials	-
HF	-	Siliceous materials	-

ADAPTED FROM—Standard Methods for the Examination of Water and Wastewater, ed. 1992. 3030D. Preliminary Digestion of Metals.

4.2.3 Method 04.12-C Dry Ash Sample Digestion for Plant Nutrients.

4.2.4 Method 04.12-E Aqua Regia Procedure.

4.3 *Closed System Digest*—this method uses closed vessels during digestion that prevent volatile losses of compounds.

4.3.1 Method 04.12-A Microwave-Assisted Nitric Acid Digestion of Compost.

4.4 *Method 04.12-A Microwave-Assisted Nitric Acid Digestion of Compost*—A representative sample of up to 0.5 g is digested in 10 mL of concentrated HNO_3 for 10 min using microwave heating with a suitable laboratory microwave unit. The sample is placed in a Teflon PFA vessel with 10 mL of concentrated HNO_3. The vessel is capped and heated in the microwave unit. After cooling, the vessel contents are diluted to volume and analyzed by the appropriate SW-846 method.

Table 04.12-2 Applicable elements for the 3051 method.

Aluminum	Copper	Selenium
Antimony	Iron	Silver
Arsenic	Lead	Sodium
Barium	Magnesium	Strontium
Beryllium	Manganese	Thallium
Boron	Mercury‡	Vanadium
Calcium	Molybdenum	Zinc
Cadmium	Nickel	
Chromium	Potassium	
Cobalt	Phosphorus	

4.4.1 The method is fast and has less opportunity for contamination than 04.12-B and 04.12-C because digestion is performed in a sealed Teflon vessel rather than an open glass beaker on a hotplate. The closed Teflon vessel minimizes chances of sample loss and contamination through volatilization or spattering.

4.4.2 The US EPA SW-846 method 3051 applies to sediments, sludges, soils, and oils and is approved by US EPA as an alternative method to SW-846 method 3050B. This method is intended to provide a rapid multi-element leach digestion prior to analysis so that decisions can be made about site cleanup levels, the need for TCLP testing of a waste, and whether a BDAT process is performing as required. It is applicable to the following elements including mercury (Hg) which is not applicable in open digest methods, (e.g., 3050B, etc.).

4.5 *Method 04.12-B Nitric Acid Digestion of Compost and Soils*—This method is an acid digestion procedure used to prepare compost samples for analysis. Digests produced by the method are suitable for analysis by ICP-AES, ICP-MS, flame atomic absorption (FLAA), and/or graphite furnace atomic absorption (GFAA). Refer to Table 04.12-4.

Table 04.12-4 Applicable metal determinations by detection method for use with the nitric acid digestion procedure, US EPA 3050B Modified.

FLAA/ICP-AES		GFAA/ICP-MS
Aluminum	Magnesium	Arsenic
Antimony	Manganese	Beryllium
Barium	Molybdenum	Cadmium
Beryllium	Nickel	Chromium
Cadmium	Potassium	Cobalt
Calcium	Silver	Iron
Chromium	Sodium	Lead
Cobalt	Thallium	Molybdenum
Copper	Vanadium	Selenium
Iron	Zinc	Thallium
Lead		

4.6 *Method 04.12-C Dry Ash Sample Digestion for Plant Nutrients*—Sample is dry ashed, treated with HNO_3, and dissolved in HCl. This method is an acid digestion procedure used to prepare the ash of compost and plant tissue samples for analysis by atomic absorption (AA) or analysis by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Table 04.12-5 Applicable metal determinations by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) detection for use with the dry ash acid digestion procedure.

Boron	Iron	Potassium
Calcium	Magnesium	Phosphorus
Copper	Manganese	Zinc

4.7 *Method 04.12-D Water Soluble Elements*—A slurry (1:20 solids:liquid ratio) is created using one part as-received compost and five equivalent parts of deionized water (17 MO·cm or purer). The 200 mL sample is shaken at a rate of 180 excursions per minute for 20 min. The liquid is separated by centrifuge (at 8000 *g* for 15 min) and passed through a 0.45 µm membrane filter. The water-soluble element content of

the filtrate is determined by ICP-AES on the filtrate and reported on a dry weight basis.

4.8 *Method 04.12-E Aqua Regia Procedure*—This method is an acid digestion procedure used to prepare compost samples for analysis. Digests produced by the method are suitable for analysis by ICP-AES, ICP-MS, and/or flame atomic absorption (FLAA), and graphite furnace atomic absorption (GFAA). Refer to Table 04.12-6.

Table 04.12-6 Applicable metal determinations by detection method for use with the Aqua Regia Procedure.

FLAA/ICP-AES		GFAA/ICP-MS
Aluminum	Magnesium	Arsenic
Antimony	Manganese	Beryllium
Barium	Molybdenum	Cadmium
Beryllium	Nickel	Chromium
Cadmium	Phosphorus	Cobalt
Calcium	Potassium	Iron
Chromium	Silver	Lead
Cobalt	Sodium	Molybdenum
Copper	Thallium	Thallium
Iron	Vanadium	
Lead	Zinc	

5. Significance and Use

5.1 Acid digestion of a sample reduces interference by dissolving chelated metals and other organo-metallic complexes. The free metal forms and corresponding concentrations within a digest solution are determined by FLAA or ICP-AES. Digestion should always be complete. Nitric acid will digest most samples adequately. Some materials require the addition of perchloric, hydrochloric, hydrofluoric or sulfuric acid for complete digestion.

5.2 *Applications of Digests for Sample Materials:*

5.2.1 *Feedstocks*—Sample preparation for feedstock analysis should comply with procedures outlined under *Method 02.02-F—Modifications for Feedstock Sample Preparation*. Special attention must be given to feedstock texture and sample uniformity. For this analysis a large sample is collected, subdivided and milled. The aliquot used in the digest representing the feedstock in question is very small, between 0.5 and 5 g.

5.2.2 *Biosolids*—Sludge materials and other similar biosolids are collected using procedures outlined in "Test Methods for Evaluating Solid Waste", Physical/Chemical Methods, SW-846, 3rd Edition, Proposed Update II, Revision I, November 1992.

5.2.3 *Compost*—Digestion methods outlined in this manual are modified for compost analysis.

5.3 *Applications:*

5.3.1 *Method 04.12-A Microwave-Assisted Nitric Acid Digestion of Compost*—This procedure is based upon US EPA SW846 Method 3051 approved for biosolids and composts containing biosolids. It is approved for use in digesting samples for determining all metals regulated under US EPA 40 CFR 503.

5.3.2 *Method 04.12-B Nitric Acid Digestion of Compost and Soils*—This procedure is based upon US EPA SW846 Method 3050 approved for biosolids and composts containing biosolids. It is approved for use in digesting samples for determining most metals regulated under US EPA 40 CFR 503, except Hg.

5.3.3 *Method 04.12-C Dry Ash Sample Digestion for Plant Nutrients*—A common procedure for the determination of plant nutrient content. It is not approved for use in digesting samples for determining metals in materials that are regulated under US EPA 40 CFR 503.

5.3.4 *Method 04.12-D Water Soluble Elements*—A common procedure for the determination of non-volatile water-soluble elements of compost does not exist. This manual provides a suite of methods to determine relative totals for elements using acid digestions. The water-soluble method is presented to provide data that represents the opposite end of this spectrum, i.e., quantities of elements most readily released or most loosely sorbed by compost solids. It is appropriate to evaluate test results as ratios in conjunction with elemental totals as determined using an acid digestion procedure.

5.3.5 *Method 04.12-E Aqua Regia Procedure*—This method has been used in many research and soils analysis laboratories and is included here for use by research and other facilities that need to use it to compare with otherwise specified regulatory methods. This procedure is not approved for use in digesting samples for determining metals in materials that are regulated under US EPA 40 CFR 503.

6. Interference and Limitations

6.1 *Method 04.12-A Microwave-Assisted Nitric Acid Digestion of Compost*—Very reactive or volatile materials that create high pressures when heated may cause venting of the vessels with potential loss of sample. Complete decomposition of either carbonates or carbon-based samples may cause enough pressure to vent the vessel. This is more likely to happen with samples larger than 0.25 g when placed in vessels (120 mL) with pressure relief devices with an upper limit of 7.5 ± 0.7 atm (110 ± 10 psi).

NOTE 2—Recommendation for compost work. Use vessels with a 200 psi pressure relief device.

6.2 *Method 04.12-B Nitric Acid Digestion of Compost and Soils*—Elements bound in silicate structures are not normally dissolved by this procedure.

If absolute total digestion is required use USEPA Method 3052. Volatile elements will be lost during the digestion process. Use Method 04.12-A, a closed vessel technique is volatile elements are to be measured.

6.3 *Method 04.12-C Dry Ash Sample Digestion for Plant Nutrients*—Volatile losses with this method may be significant for elements such as Hg, As, and Se with composted materials high in organo-metallic complexes.

6.4 *Method 04.12-D Water Soluble Elements*—The clay content of most composts is insignificant. If present, clay can bind elements and decrease the concentration of water-soluble elements.

6.5 *Method 04.12-E Aqua Regia Procedure*—Used to extract total metals from the soil. This method is not quite as total as use of HF, but it has been shown to reliably extract total metals from contaminated soils. Use of pressure digestion vessels (CEM) with HNO_3 or Aqua Regia would be a slightly better procedure. This digestion procedure is not approved for use in digesting samples for determination of elements in materials that are regulated under US EPA 40 CFR 503.

7. Sample Handling

7.1 A sampling plan that addresses the considerations discussed in *02.01-C Sampling Plan for Composted Material* shall be followed to collect samples.

7.2 Sample containers must be prewashed with detergents, (e.g., 10% RBS, P-free and B-free), acids, and Type II water. Plastic and glass containers are suitable. Refer to Chapter 3 in Annual Book of ASTM Standards for detailed information.

7.3 Samples must be refrigerated (4°C) upon receipt and analyzed as soon as possible.

7.4 *Method 04.12-A Microwave-Assisted Nitric Acid Digestion of Compost*—Use material free of inerts > 2-mm, that is finely milled and passes a 0.5-mm (35-mesh) sieve. Refer to Method 02.02-E.

7.5 *Method 04.12-B Nitric Acid Digestion of Compost and Soils*

7.5.1 *Option One*—Use one to three grams of as-received, moist, unmilled, sample material that is free of inerts > 2-mm, and passes a 9.5-mm sieve. Refer to Method 02.02-B.

7.5.2 *Option Two*—Use one to three grams of air-dried, milled material that is free of inerts > 2-mm, finely milled to pass a 0.5-mm (35-mesh) sieve. Refer to Method 02.02-E.

7.6 *Method 04.12-C Dry Ash Sample Digestion for Plant Nutrients*—Use an oven-dried (70±5°C) material free of inerts > 4-mm, that is finely milled and passes a 0.5-mm (35-mesh) sieve. Store milled compost sample in a sealed plastic or glass container at ambient laboratory temperature (~23°C). Refer to Method 02.02-E.

7.7 *Method 04.12-D Water Soluble Elements*—Use as-received moist material free of inerts > 4 mm, that passes a 9.5 mm sieve. Use refrigerated storage (4°C) for the sieved compost sample. Seal the sample in a prewashed (acid and Type II water) plastic or glass container. Refer to Method 02.02-B.

7.8 *Method 04.12-E Aqua Regia Procedure:*

7.8.1 *Option One*—Use one to three grams of as-received, moist, unmilled, sample material that is free of inerts > 2-mm, and passes a 9.5-mm sieve. Refer to Method 02.02-B.

7.8.2 *Option Two*—Use one to three grams of air-dried, milled material that is free of inerts > 2-mm, finely milled to pass a 0.5-mm (35-mesh) sieve. Refer to Method 02.02-E.

Test Method: Digestion Techniques: Microwave Assisted Nitric Acid Digestion for Compost, *adapted from SW-846 US EPA Method 3051*							**Units:** *NA*	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.12-A	04.12-A						04.12-A	04.12-A

04.12-A MICROWAVE ASSISTED NITRIC ACID DIGESTION FOR COMPOST

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

ADAPTED FROM— SW-846 US EPA Method 3051. Suggested modifications to 3051A were identified and outlined by William S. Dancer, Ph.D.; Research Analytical Laboratory, Department of Soil, Water and Climate, University of MN, St. Paul.

8. Apparatus for Method A

8.1 *Microwave Digestion System*—(e.g., CEM Model MDS-2000. CEM Corporation, Box 200, Matthews, NC 28106).

8.2 *Microwave Digestion Vessels*—with a minimum operating temperature of 200°C and minimum operating pressure of 200 psi, CEM digestion vessels, with Teflon PFA® liners or equal.

8.3 *Microwave Apparatus Requirements:*

8.3.1 CEM MDS-2000 microwave unit provides power that can be programmed to within ±10W of the 574 W required by the 3051 procedure.

8.3.2 Microwave unit cavity is corrosion resistant as well as ventilated.

8.4 *Electrical Components*—Protected against corrosion for safe operation.

8.4.1 System requires Teflon PFA digestion vessels (~120 mL capacity) capable of withstanding pressures up to 15.3 ± 1.7 atm (225 ± 25 psi) and capable of controlled pressure relief at pressures exceeding 17.0 atm (250 psi).

8.4.2 A rotating or oscillating turntable is employed to ensure homogenous distribution of microwave radiation within the unit. The speed of the turntable should be a minimum of 3 rpm.

8.4.3 Laboratory-grade microwave digestion equipment is required to prevent safety hazards; laboratories using or contemplating the use of non-laboratory microwave ovens and Teflon containers for this method should be aware of safety requirements.

8.4.3.1 *Use unit with corrosion resistant electrical components and safety devices*—When an acid such as HNO_3 is used to assist sample digestion in microwave units with open or vented vessels, acid gases are released that can corrode safety devices that prevent the microwave magnetron from engaging when the microwave oven door is opened. This will result in operator exposure to microwave energy.

8.4.3.2 *Unlined PFA Teflon Containers with Pressure Relief Mechanisms.* Temperature is the important variable controlling the reaction. Pressure is needed to attain elevated temperatures and must be safely contained. Many digestion vessels constructed from certain types of Teflon may crack, burst, or explode in the unit under high pressure. The 3051 method requires pressure resistant vessels with Teflon liners that can withstand pressures of 13.6 atm (200 psi) and temperatures of 200°C. The PFA unlined containers have maximum operating pressures of about 100°C and pressure of (6.8 atm) 100 psi; i.e., vessels used by the MDS-81D system produced in 1989.

8.5 *Polymeric Volumetric Ware in Plastic*—Teflon or polyethylene, 50- or 100-mL capacity.

8.6 *Filter Paper*—Whatman No. 41 or equivalent, unless samples are allowed to settle followed by centrifugation.

8.7 *Filter Funnel*—disposable polypropylene, if samples are filtered.

8.8 *Analytical Balance*—300 g capacity, with accuracy of ± 0.001 g.

9. Reagents for Method A

9.1 To minimize background levels due to metallic contamination all acids should be sub-boiling and distilled. Other grades may be used, provided it is ascertained that the reagent is of adequate purity for use and does not diminish the accuracy of the determination.

9.2 *Nitric Acid*—70%, purified and certified for trace element analyses, (e.g., J.T. Baker "InstraAnalyzed" grade). Acid type or brand is not specified by the US EPA.

9.3 *Reagent Water*—Deionized 17 MΩ·cm purity.

10. Procedure for Method A

10.1 *Calibrate Microwave Equipment:*

10.1.1 Measurement of the available power for heating is evaluated so that absolute power in W may be transferred from one microwave unit to another. For cavity-type microwave equipment, this is accomplished by measuring the temperature rise in 1 kg of water exposed to microwave radiation for a fixed period of time. The analyst can relate power in W to the partial power setting of the unit. The calibration formation required for laboratory microwave units depends upon the type of electronic system used by the manufacturer to provide partial microwave power. Few units have an accurate and precise linear relationship between percent power settings and absorbed power. Where linear circuits are utilized, the calibration curve is determined by a three-point calibration method, SW-846 Volume One, Section A, Part 1, otherwise, the analyst must use the multiple-point calibration method.

10.1.2 The multiple-point calibration involves the measurement of absorbed power over a large range of power settings. Typically, for a 600 W unit, the following power settings are measured: 100, 99, 98, 98, 95, 90, 80, 70, 60, 50, and 40% using the procedure described in Method 10.14. This data is clustered about the customary 574 Watt working power range. Non-linearity was commonly encountered at the upper end of the calibration. If the unit's electronics is known to have nonlinear deviations in any region of proportional power control, it will be necessary to make a set of measurements that bracket the power to be used. The final calibration point should be at the partial power setting that will be used in the test. This setting should be checked periodically to evaluate the integrity of the calibration. If a significant change is detected (± 10 W), then the entire calibration should be re-evaluated.

10.1.3 The three-point calibration involves the measurement of absorbed power at three different power settings. Measure the power at 100% and 50% using the procedure described in Step 10.1.4 (below). From the two-point line calculate the power setting corresponding to the required power in W specified in the procedure. Measure the absorbed power at that partial power setting. If the measured absorbed power does not correspond to the specified power within ± 10 W, use the multiple point calibration (Step 2, above). This point should also be used to periodically verify the integrity of the calibration.

10.1.4 Equilibrate a large volume of water to room temperature (~23°C). One kg of reagent water is weighed (1,000.0 g ± 0.1 g) into a Teflon beaker or a beaker made of some other material that does not significantly absorb microwave energy (glass absorbs microwave energy and is not recommended). The initial temperature of the water should be 23±2°C measured to ±0.05°C. Use a new sample for each additional measurement. If the water is reused both the water and the beaker must have returned to 23±2°C. Three measurements at each power setting should be made.

10.1.5 The absorbed power is determined by the following relationship:

$$P = (K \times Cp \times m \times ?T) \div t \qquad \textit{Equation 10.1.5}$$

where:

P = apparent power absorbed by sample in W, W = joule sec^{-1},
K = conversion factor for thermochemical calories sec^{-1} to W = 4.184,
Cp = heat capacity, thermal capacity, or specific heat of water, cal $g^{-1}\cdot°C^{-1}$,
m = mass of water sample in grams, g,
?T = final temperature minus initial temperature, °C, and
t = time in seconds, s.

10.1.6 For 1 kg of distilled water (heat capacity at 25°C is 9.9997 cal $g^{-1}\cdot°C^{-1}$) heated for 2 min the calibration equation becomes:

$$P = T \times 34.85 \qquad \textit{Equation 10.1.6}$$

NOTE A2—Stable line voltage is necessary for accurate and reproducible calibration and operation. The line voltage should be within manufacturer's specification, and during measurement and operation should not vary by more than ± 2 V. A constant power supply may be necessary for microwave use if the source of the line voltage is unstable.

10.1.7 Electronic components in most microwave units are matched to the unit's function and output. When any part of the high voltage circuit, power source, or control components in the unit are serviced or replaced, it will be necessary to recheck the units' calibration. If the power output has changed significantly (± 10 W), then the entire calibration should be re-evaluated.

10.2 *Microwave Digestion Vessel Cleaning:*

10.2.1 All digestion vessels and volumetric ware must be carefully acid washed and rinsed with reagent water. When switching between high and low concentration samples, all digestion vessels should be cleaned by leaching with hot (1:1) hydrochloric acid for a minimum of 2 h followed with hot (1:1) HNO_3 for a minimum of 2 h and rinsed with reagent water and dried in a clean environment. This cleaning procedure should be used when the prior contents of the digestion vessel are unknown or cross contamination from vessels is suspected. Polymeric volumetric ware and storage containers should be cleaned with dilute acids appropriate for the specific plastics used and rinsed with reagent water and dried in a clean environment.

NOTE A1—It is advantageous to keep liners used for lower levels of trace metals separate from those used for higher levels of metals. Routinely clean liners by soaking in 5 to 10 % HCl for 4 h followed by microwave heating for 15

min at 650 W with ~3-mL of (1:1) HNO_3. Also, routinely presoak Teflon liners in cleaner, (e.g., Micro® or MicroClean™), for 4 h before soaking them in HCl.

10.3 *Sample Digestion:*

10.3.1 Weigh the Teflon PFA digestion vessel, valve, and cap assembly to ±0.001 g prior to use.

10.3.2 Weigh 0.25 g of mixed and ground compost sample to the nearest 0.001 g into the Teflon PFA sample vessel equipped with a single ported cap and a pressure relief valve. For soils, sediments, and sludges use no more than 0.500 g. For oils use no more than 0.250 g.

NOTE A3—The original EPA method does not call for grinding the sample.

10.3.3 Add 10 ± 0.1 mL concentrated HNO_3 in a fume hood. If a vigorous reaction occurs, allow the reaction to stop before capping the vessel. Cap the vessel and torque the cap to 12 ft lb or according to the unit manufacturer's directions. The sample vessel may be connected to an overflow vessel using Teflon PFA connecting tubes. Weigh the vessels to the nearest 0.001 g. Place vessels in the microwave carousel. Connect the overflow vessels to the center well of the unit.

CAUTION—When digesting samples containing volatile or easily oxidized organic compounds, initially weigh no more than 0.10 g and observe the reaction before capping the vessel. If a vigorous reaction occurs, allow the reaction to cease before capping the vessel. If no appreciable reaction occurs, a sample weight up to 0.25 g can be used.

10.3.4 Place the vessels in the turntable of the microwave in groups of two or 6-sample vessels. The vessels are evenly distributed on the turntable. Any vessels containing 100 mL of HNO_3 for analytical blank purposes are counted as sample vessels. When fewer than the recommended number of samples are to be digested, (i.e., 3 samples plus 1 blank), the remaining vessels should be filled with 10 mL of HNO_3 to achieve the full complement of vessels. This provides an energy balance since the microwave power absorbed is proportional to the total mass in the cavity. Irradiate each group of 2-sample vessels at 344 W for 10 min and each group of 6 sample vessels at 574 W for 10 min. The temperature of each sample should rise to 175°C in less than 5.5 min and remain between 170 and 180°C for the balance of the 10 min irradiation period. The pressure should peak at less than 6 atm for most compost samples. The pressure will exceed these limits in the case of high concentrations of carbonate or organic compounds. In these cases the pressure will be limited by the relief pressure of the vessel to 15.3 ± 1.7 atm (225 ± 25 psi).

10.3.4.1 *Capacity*—Newer microwave units may be capable of higher power (W) that permits digestion of a larger number of samples per batch. If the analyst wishes to digest more than two or six samples at a time, the analyst may use different values of power as long as they result in the same time and temperature conditions defined in step 10.3.4. That is, any sequence of power that brings the samples to 175°C in 5.5 min and permits a slow rise to 175-180°C during the remaining 4.5. min.

10.3.5 At the end of the microwave program, allow the vessels to cool for a minimum of 5 min before removing them from the microwave unit. When the vessels have cooled to room temperature (~23°C), weigh and record the weight of each vessel assembly. If the weight of acid plus sample has decreased by more than 10% from the original weight, discard the sample. Determine the reason for the weight loss. These are typically attributed to loss of vessel seal integrity, use of a digestion time longer than 10 min, too large a sample, or improper heating conditions. Once the source of the loss is corrected, prepare a new sample or set of samples.

10.3.6 Weigh each vessel assembly. If sample weight decreases by more than 10%, significant loss of material has occurred; correct the loss problem and repeat the digestion process following steps 10.3.1 through 10.3.5.

10.3.7 Complete the preparation of the sample by carefully uncapping and venting each vessel in a fume hood. Transfer the sample to an acid cleaned polyethylene bottle. Dilute the digest and sample standards to a known volume. If the digested sample contains particulate matter that may clog nebulizers or interfere with injection of the sample into the instrument, the sample may be centrifuged, allowed to settle, or filtered.

10.3.7.1 *Centrifugation*—Centrifugation at 2,000-3,000 rpm for 10 min is usually sufficient to clear the supernatant.

10.3.7.2 *Settling*—Allow the sample to stand until the supernatant is clear. This is usually accomplish if the sample is left overnight. If it does not, centrifuge or filter the sample.

10.3.7.3 *Filtering*—The filtering apparatus must be thoroughly cleaned and prerinsed with dilute HNO_3. Filter the sample through quantitative filter paper into a second acid-cleaned container.

10.3.8 The digest is now ready for analysis of metals using the appropriate SW-846 method.

10.4 *Spectrochemical Analysis:*

10.4.1 Mercury is determined on the Method 3051A sample digest with cold vapor atomic absorption spectrophotometry following US EPA Methods 7470A or 245.1. See *Test Method 04.12-B Cold Vapor Technique for Compost* for detailed description of procedure.

10.4.2 Other elements are determined by US EPA Method 6010A, inductively coupled plasma atomic emission spectrometry. Includes: Ca, Mg, Na, K, P, Fe, Al, Mn, B, Cu, Zn, Cu, Pb, Cd, Cr, Ni, Mo, and As.

11. Corrections for Method A

11.1 Convert the concentration measured on an air dried sample (36°C) to oven dw (70±5°C).

12. Modifications and Exceptions for Method A

12.1 Method 3051 is applied as published by the US EPA with the exception of the basis of reporting. The method calls for reporting on the basis of the sample weighed for the test. It is recommended that a compost sample is air dried at 36°C to minimize Hg loss through volatilization. Also, metal concentrations are reported on a 70±5°C basis. Plant analysis methods commonly call for drying and reporting within a range of 60-70°C. The US EPA in a 1973 publication recommended a drying temperature of 65-75°C. This text closely follows that of the 1973 US EPA publication.

Test Method: Digestion Methods. Nitric Acid Digestion of Compost and Soils, *adapted from SW-846 US EPA Method 3050*							**Units:** *NA*	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.12-B	04.12-B						04.12-B	04.12-B

04.12-B NITRIC ACID DIGESTION OF COMPOST AND SOILS

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

ADAPTED FROM—SW-846 US EPA Method 3050.

13. Apparatus for Method B

13.1 *Digestion Tubes*—250-mL, with reflux capabilities, (e.g., inverted small glass funnels in the tubes to allow refluxing).

13.2 *Block Digester.*

13.3 *Filter Funnels*.

13.4 *Graduated Cylinder.*

13.5 *Thermometer*—range of 0°C to 200°C.

13.6 *Analytical Balance*—accurate to 0.001 g.

14. Reagents and Materials for Method B

NOTE 1B—To minimize background levels due to metallic contamination all acids should be sub-boiled and distilled. Other grades may be used, provided it is ascertained that the reagent is of adequate purity for use and does not diminish the accuracy of the measurement.

14.1 *Reagent Water*—Type II deionized, minimum resistivity 17 MO·cm (minimum standard); water should be monitored for impurities.

14.2 *Concentrated Nitric Acid, reagent grade (HNO_3)*—Acid should be analyzed to determine level of impurities. If method blank < MDL, the acid can be used.

14.3 *Concentrated Hydrochloric Acid, reagent grade (HCl)*—Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

14.4 *Hydrogen Peroxide (30%, H_2O_2)*—Oxidant should be analyzed to determine level of impurities.

14.5 *Filter Paper—(e.g., Whatman 41* or equivalent).

15. Procedure for Method B

15.1 *Sample Weight*—Mix sample thoroughly to achieve homogeneity. Use either sieved, as-received moist material, or air-dried, milled material. Refer to paragraph 7.5 of Sampling Handling for details. Weigh 1 to 3 g of sample to the nearest 0.01 g and transfer to a 250 mL volumetric block digestion tube. Determine total solids content of a separate aliquot.

15.2 *Reflux Sample*—Add 10 mL of 1:1 HNO_3 and mix the slurry well. Set controller on block digester to reflux samples at 95°C for 15 min. Add 5 mL concentrated nitric acid (HNO_3), and reflux at 95°C for 30 min without boiling. After 30 min, add 3 mL of concentrated hydrochloric acid (HCl) and continue to heat for 1 h or as needed to reduce volume to 10 mL.

15.3 *Add H_2O_2*—After the digestion block cools to approximately 80°C, *very slowly* add 3 mL of 30% H_2O_2 and heat until effervescence subsides. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. If substantial effervescence occurs, very slowly continue to add 1 mL increments of 30% H_2O_2 and heat until effervescence subsides. At no time add more than 10 mL total of 30% H_2O_2. Add 10 mL of reagent water and reflux at 95°C for 15 min.

15.4 *Cool Sample*—Turn off block digester controller and remove samples from block. Allow samples to cool and wash down the beaker walls with water. Filter into a 100-mL volumetric flask with filter and adjust final volume to 100 mL with reagent water.

15.5 *Run Parallel Spikes, Blanks and Replicates*—For each batch of samples treated by this method run a blank and a blank spike (10 ng L^{-1} for GFAA metals, 1 mg L^{-1} for FLAA metals). For every 10 samples run a duplicate, a matrix spike (10 ng L^{-1} for GFAA metals, 1 mg L^{-1} for FLAA metals), and a matrix spike duplicate.

16. Corrections for Method B

16.1 Report elemental concentrations on an oven dry weight basis.

Test Method: Digestion Techniques. Dry Ash Sample Digestion for Plant Nutrients, *adapted from AOAC 985.01*							**Units:** *NA*	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.12-C	04.12-C							04.12-C

04.12-C DRY ASH SAMPLE DIGESTION FOR PLANT NUTRIENTS

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

ADAPTED FROM—AOAC Method 985.01.

17. Apparatus for Method C

17.1 *Crucible with Cover*—high-form glazed porcelain, or silica-glass.

17.2 *Analytical Balance*—accurate to ±0.001 g.

17.3 *Muffle Furnace*—heated to 500±50°C.

17.4 *Desiccation Chamber and Desiccant*.

17.5 *Digestion Vial*.

17.6 *Volumetric Pipettes*—1, 5, 10, 20 50 mL (TD).

17.7 *Volumetric Flasks*—50-mL and 1.0-L.

18. Reagents and Materials for Method C

18.1 *Concentrated Nitric Acid, (HNO_3)*—Reagent grade acid should be analyzed to determine level of impurities. If method blank < MDL, the acid can be used.

18.2 *Concentrated Hydrochloric Acid (HCl)*—Reagent grade acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

18.3 *Reagent Water*—Type II deionized, minimum resistivity 17 MO·cm (minimum standard); water should be monitored for impurities.

18.4 *Stock Solutions (1,000 µg mL^{-1})*—Weigh designated reagent into separate 1-L volumetric flasks. Dissolve in minute amount of dissolving reagent. Dilute to volume with H_2O.

Table 04.12-C1 Stock solutions for use with the dry ash acid digestion procedure.

Element	*Reagent*	*g*	*Dissolving Reagent*
B	H_3BO_3	5.7192	H_2O
Ca	$CaCO_3$	2.4973	6 *N* HCl
Cu	Pure metal	1.0000	HNO_3
K	KCl	1.9067	H_2O
Mg	$MgSO_4 \cdot 7H_2O$	10.1382	H_2O
Mn	MnO_2	1.5825	6 *N* HCl
P	$NH_4H_2PO_4$	3.7138	H_2O
Zn	Pure metal	1.0000	6 *N* HCl

18.5 *Standard Solutions*—Pipette the following volumes of stock solution into 1 L volumetric flasks. Add 100 mL HCl and dilute to volume with H_2O. Make any subsequent dilutions with 10% HCl (1+9 dilution).

Table 04.12-C2 Standard solutions for use with the dry ash Acid digestion procedure.

Element	*Standard Solution 1*		*Standard Solution 2*	
	Stock Solution (mL)	*Final Concentration (µg mL^{-1})*	*Stock Solution (mL)*	*Final Concentration (µg mL^{-1})*
B	0	0	10	10
Ca	5	5	60	60
Cu	0	0	1	1
K	5	5	60	60
Mg	1	1	20	20
Mn	0	0	10	10
P	5	5	60	60
Zn	0	0	10	10

18.6 *Suggested operating parameters for ICP emission spectrometer*—forward power of 1.1 kW; reflected power < 10 W; aspiration rate of 0.85-3.5 mL min^{-1}; flush between samples of 15-45 s; integration time of 1-10 s.

Table 04.12-C3 ICP wavelength table of elements for the dry ash acid digestion procedure.

Element	*Wavelength (nm)*
B (CAS-7440-42-8) †	249.67
Ca (CAS-7440-70-2)	317.93
Cu (CAS-7440-50-8)	324.75
K (CAS-7440-09-7)	766.50
Mg (CAS-7440-95-4)	279.80
Mn (CAS-7440-96-5)	257.61
P (CAS-7440-14-0)	214.92
S (CAS-7704-34-9)	180.67
Zn (CAS-7440-66-6)	213.85

†CAS is the Chemical Abstract Service number

19. Procedure for Method C

19.1 *Weigh Sample*—Accurately weigh 1 g sample, ground and oven dried at 70±5°C into glazed, high form porcelain crucible.

19.2 *Ash Sample*—Ash the crucible containing weighed sample in muffle furnace (550°C) for 2 h; cool crucible with ashed sample in desiccator for approximately 1 h.

19.3 *Wet Ash*—Wet ash with 10 drops (mL) of H_2O and add 3-4 mL 50% HNO_3 (1+1). Evaporate excess HNO_3 on hot plate set at 100-120°C.

19.4 *Re-ash Sample*—Return crucible to muffle furnace and ash at 550°C for additional 1 h at 550°C . Cool crucible in desiccator for approximately 1 h.

19.5 *Acid Digest*—Dissolve ash in 10 mL HCl (1+1) and transfer quantitatively to 50 mL volumetric flask. Dilute to volume with H_2O.

19.6 *ICP Elemental Determination*—Elemental determination is accomplished by ICP emission spectroscopy. Calibration of instrument is conducted with standards. After calibration is complete, samples are analyzed. Check calibration every 10 samples. If instrument drifts out of calibration (> 3% of original values), repeat calibration steps.

Test Method: Digestion Techniques. Water-Soluble Elements.						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.12-D	04.12-D	04.12-D		04.12-D				04.12-D

04.12-D WATER-SOLUBLE ELEMENTS

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

20. Apparatus for Method D

20.1 *Analytical Balance*—accurate to ±0.001 g.

20.2 *Centrifuge Extraction Apparatus*—200-mL centrifuge tubes, capable of withstanding 8000 *g*.

20.3 *Glass Fiber Filter*

20.4 *Membrane Filter*—0.45 µm with assembly.

20.5 *Sample Containers*—500-mL Erlenmeyer-type, fitted with screw-cap or other secure cover.

20.6 *Shaker*—reciprocating or end-over-end shaker capable of shaking a sample flask at the rate of 180 reciprocations or excursions per min.

21. Reagents and Materials for Method D

21.1 *Reagent Water*—Deionized, minimum resistivity of 17 MΩ·cm (minimum standard). Water should be monitored for impurities.

22. Procedure for Method D

22.1 *Compost Aliquot Moisture*—Determine the total solids ratio on a parallel sample aliquot of the test aliquot.

22.2 *Prepare Samples:*

22.2.1 Transfer 20.0 g of as-received moist compost into a sample container.

22.2.2 Add 400 mL of deionized water to the as-received moist compost aliquot.

22.2.3 Shake the the 1:20 slurry sample for 20 min at 180 reciprocations or excursions per minute.

22.2.4 Maintain slurry at ambient laboratory temperature, (e.g., 20-23°C).

22.3 *Extraction*

22.3.1 Transfer the free liquid fraction of the slurry to a 200-mL centrifuge tube. Centrifuge at 8000 *g* for fifteen min.

22.4 *Membrane Filtration*

22.4.1 *Optional*—Filter centrifiguate though a glass fiber filter to prepare it for 0.45 µm membrane filtration.

22.4.2 Filter approximately 30 mL of the centrifugate through a 0.45 µm membrane filter into a plastic disposable ICP tube.

22.5 *ICP Elemental Determination*—Elemental determination is accomplished by ICP emission spectroscopy. Calibration of instrument is conducted with standards. After calibration is complete, samples are analyzed. Check calibration every 10 samples.

22.5.1 Report elemental concentration on a dry weight basis.

NOTE 1E—Quantities of elements by species is not covered with this method.

Test Method: Digestion Techniques. Aqua Regia Procedure.						**Units:** *NA*		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.12-E	04.12- E						04.12- E	04.12- E

04.12-E AQUA REGIA PROCEDURE

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

23. Apparatus for Method E

23.1 *Beakers*—soil analysis 200 mL tall form, with watch glass.

23.2 *Volumetric Flasks*—50 mL.

23.3 *Counting Vial*—20 mL.

23.4 *Hot Plate.*

23.5 *Repipets*—5 mL, 15 mL, and 20 mL.

23.6 *Safety Equipment*—hand and eye protection, labcoat, and plastic apron.

24. Reagents and Materials for Method E

24.1 *Filter Paper*—#40 Whatman™, and prefolded filter papers, (e.g., Ahlstrom Filtration, Inc. Grade 513-Fluted).

24.2 *Concentrated Nitric Acid, (HNO_3)*—Reagent grade acid should be analyzed to determine level of impurities.

24.3 *Concentrated Hydrochloric Acid (HCl)*—Reagent grade acid should be analyzed to determine level of impurities.

24.3.1 0.1 *N* HCl, and 3 *N* HCl.

24.4 *Reagent Water*—Type II deionized, minimum resistivity 17 MO·cm (minimum standard); water should be monitored for impurities.

25. Procedure for Method E

25.1 Weigh out 10 g air dry samples into soil analysis 200 mL tall form beakers. Use 1 blank per 10 samples (no fewer than 3 with any batch of samples). Use 1 reference blank per 10 compost samples (no fewer than 3 per batch of samples). Randomly run every fifth sample in duplicate. With every batch of samples, also run reference biosolids or compost samples with known composition to check reliability of analysis (e.g., CAP samples, or an appropriate NIST standard).

Caution!—Aqua Regia is a very strong acid mixture which can even dissolve gold. Wear hand and eye protection, labcoat, and possibly the plastic apron.

25.2 *Acid*—Wet the compost aliquot and add ONLY the nitric acid and SLOWLY start to heat the beakers. After the initial nitric has been used and evaporated off, then proceed.

25.2.1 Place the beakers on a COLD hot plate or let set for a while before placing on a HOT hot plate; organic soils will rapidly react and self-heat, so water may be added to slow the initial reaction. Place a watch glass on the beaker.

25.2.2 Put the hot plate on the higher marked setting (a temperature that is high enough to cause evaporation, but not high enough for boiling and the popping of the watch glass) for approximately a total of 2 h. Stir the samples after they have started warming until the entire compost sample is wetted/suspended. Stir them again after 1 h. After two hours, place the watch glass ajar, about half open, and allow to boil to near dryness.

25.3 *Reflux Samples*—Add 20 mL 3 *N* HCl to each beaker, cover with same watch glass, and place on hot plate at lower mark setting for 2 h (a temperature that causes refluxing - a dripping of liquid off the watch glass - but not boiling of the solution); stir the beakers intermittently to assure the compost residue is suspended.

25.4 *Filter the Samples*—After acid extraction, filter the samples using prefolded filter papers placed inside #40 Whatman filter paper. Pre-wash the filter, funnel, and 50 mL volumetric flask using 0.1 *N* HCl, and discard rinse into hazardous waste container. Pour the digestate from the beaker into filter paper; then wash the remaining material in the beaker into the filter. Collect filtrate in 50 mL volumetric flask; rinse beaker using 0.1 *N* HCl and pour into filter; after this has drained, rinse the filter paper well [starting at the top] using 0.1 *N* HCl; dilute to 50 mL using deionized water, shake well.

NOTE E1—Check before starting the sample filtering process if COBALT is to be added as an internal standard. This standard is added after the filter - volumetric flask rinse, but before the sample filtration begins.

25.5 Rinse the 20 mL counting vial with mixed solution and discard rinse into hazardous waste container; transfer 20 mL to counting vial, and discard rest into

hazardous waste container. Make sure that identification label is transferred from beaker to volumetric flask to the counting vial.

04.12 METHODS SUMMARY

26. Report

26.1 Concentrations (mg kg^{-1}) determined are reported on a 70±5°C oven dry weight basis. The US EPA method calls for reporting on the basis of the actual weight of the original sample.

26.1.1 Quality control data is to be maintained and available for reference or inspection for a period of three years, (local regulations may vary). This method is restricted to use by, or under supervision of, experienced lab analysts.

26.1.2 Replicate samples should be processed routinely. A replicate sample is a sample brought through the whole sample preparation and analytical process. A replicate sample should be processed with each analytical batch or every 20 samples whichever is the greater number.

26.1.3 Spiked samples or standard reference materials should be included with each group of samples processed or every 20 samples, whichever is the greater number. A spiked sample should also be included whenever a new sample matrix is being analyzed.

27. Precision and Bias

27.1 *Method 04.12-A Microwave-Assisted Nitric Acid Digestion of Compost:*

27.1.1 *Precision*—See Table 04.07-A6 ICP-AES precision University of Minnesota Standard Reference compost sample.

27.1.2 *Bias*—See Table 04.07-A4 Elemental Analysis by ICP-AES comparing results by US EPA Method 3051 and ICP-AES on a European Certified Industrial Sewage Sludge Reference Sample (BCR). Results for mercury and other certified and exchange samples are outlined in the method for mercury.

27.1.3 See US EPA Method 3051 and Binstock, et al. (1989)

27.2 *Method 04.12-B Nitric Acid Digestion of Compost and Soils*—The precision and bias of this test are not determined. Data are being sought for use in developing a precision and bias statement.

27.3 *Method 04.12-C Dry Ash Sample Digestion for Plant Nutrients*—The precision and bias of this test are not determined. Data are being sought for use in developing a precision and bias statement.

27.4 *Method 04.12-D Water-Soluble Elements*—The precision and bias of this test are not determined. Data are being sought for use in developing a precision and bias statement.

27.5 *Method 04.12-E Aqua Regia Procedure*—The precision and bias of this test are not determined. Data are being sought for use in developing a precision and bias statement.

28. Keywords

28.1 digest; nitric acid; nitric-perchloric acid; perchloric acid; hydrochloric acid; water-soluble; totals; Aqua Regia

Test Method: Atomic Absorption. Two Methods						**Units:** mg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.13-A	04.13-A					04.13-A	04.13-A	04.13-A
04.13-B	04.13-B					04.13-B	04.13-B	04.13-B

04.13 ATOMIC ABSORPTION SPECTROMETRY

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the determination of elemental concentrations from a prepared digest solution by use of atomic absorption spectrophotometry.

1.1.1 *Method 04.13-A Cold Vapor AAS Technique for Mercury in Compost.*

1.1.2 *Method 04.13-B Atomic Absorption Methods.*

COMMENT—This test method is presented for convenience. It is a copy of the AA method presented in SW846, formatted to conform to the style of TMECC.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *Standard Reference Manuals*

ASTM Standard Specifications for Reagent Water. 1985. In Annual Book of ASTM Standards. Vol. 11.01. Philadelphia, PA. 1985. D1193-77.

Methods for Chemical Analysis of Water and Wastes. 1983. US EPA. Office of Research and Development. Environmental Monitoring and Support Laboratory. ORD Publication Offices of Center for Environmental Research Information. Cincinnati, OH. EPA-600/4-79-020.

Rohrbough, W.G., et al., eds. 1986. Reagent Chemicals, American Chemical Society Specifications. 7th ed. 1986. American Chemical Society. Washington, DC.

US EPA Method 7000A. Atomic Absorption Methods. *In* Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, November 1992, SW-846, 3rd Edition.

2.2 *Method 04.13-A Cold Vapor AAS Technique for Mercury in Compost:*

International Manure and Refuse Sample Exchange Program. Quarterly Report 94.1, 1-3/94; and 94.2, 4-6/94. Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, The Netherlands.

Mercury in Drinking, Surface, Saline Waters, Domestic and Industrial Wastes. *In* Method for Chemical Analysis of Water and Wastes. EPA-600/4-79-020, March 1979. Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

Mercury in Solid or Semisolid Waste. *In* Test Methods for Evaluating Solid Waste. Volume 1A: Laboratory Manual-Physical/Chemical Methods, SW-846, Proposed Update II, November 1992. Environmental Monitoring and Support Laboratory, Cincinnati, OH. 45268.

Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils. *In* Test Methods for Evaluating Solid Waste. Volume 1A: Laboratory Manual-Physical/Chemical Methods, SW-846, 3rd Edition, Proposed Update II (Revision), November 1992. Environmental Monitoring and Support Laboratory, Cincinnati, OH. 45268.

Montana Soil II. Standard Reference Material (SRM) 2411. National Institute of Standards and Technology (NIST). Gaithersburg, MD 20899.

Sewage Sludge. Certified Reference Material BCR 146. Community Bureau of Reference, Brussels, Belgium.

2.3 *Method 04.13-B Atomic Absorption Spectrophotometry Methods, US EPA Method 7000A from Document SW846:*

Knopp, J.F., M.C. Longbottom and L.B. Lobring. 1972. Cold Vapor Method for the Determination of Mercury. J. Amer. Water Works Assoc. 64:20-25.

US EPA Method 245.1. Mercury Cold Vapor Manual. 1979. *In* Methods for Chemical Analysis of Water and Wastes. US EPA-600/4-79-020. March 1979.

LDC Analytical (undated) MercuryMonitor™ Elemental Mercury Detector Instruction Manual. Manual Number 870097. 29 pp. LDC Analytical, Riviera Beach Florida.

El-Awaly, A.A., R.B. Miller, and M.J. Carter. 1976. Automated method for the determination of total and inorganic mercury in water and wastewater samples. Anal. Chem. 48:110-118.

3. Terminology

3.1 *element, n*—A substance composed of atoms having an identical number of protons in each nucleus. Elements cannot be reduced to simpler substances by normal chemical means.

3.2 *nutrient, n*—A source of nourishment, especially an ingredient in food.

3.3 *standard, n*—Serving as or conforming to a standard of measurement or value. Sample often referred to a standard reference sample or check. This is because the reference sample's physical, chemical or biological characteristics are known and therefore it can be used to monitor analytical bias or accuracy of a physical, chemical or biological determination.

4. Summary of Test Methods

4.1 *Method 04.13-A Cold Vapor AAS Technique for Mercury in Compost:*

4.1.1 Compost (approximately 0.5 g) is digested with 10 mL of concentrated nitric acid following *TMECC Method 04.12-A Digest Techniques* and quantitatively transferred to a 50-mL centrifuge and brought to a 40 mL volume. A small volume of the resulting 25% nitric acid digest (0.5 mL) is diluted with water (4.5 mL) and heated in a waterbath at 95°C with half normal sulfuric acid, by potassium permanganate and potassium persulfate. After 2 h, oxidation is complete and the same sample vessel is attached to the cold vapor atomic absorption apparatus. Mercury ions are reduced to elemental mercury (Hg^0) with $SnCl_2$, and nitrogen gas is bubbled through the solution and to carry the mercury through a cell positioned in the light path of an atomic absorption spectrophotometer. Absorbance (peak height) is measured at 253.7 nm wavelength and the concentration of mercury in the sample is calculated by reference to a standard calibration graph.

4.1.2 In general, compared to water samples compost tends to contain higher concentrations of mercury; smaller volumes are analyzed (0.5 c.f. 5 mL) and additional dilution may be required to measure the Hg in compost samples depending upon the precision of the cold vapor AA used.

4.1.3 The minimum detection level of mercury in compost extracts by cold vapor AA is about 70 ng mL^{-1} (ppb) as compared to about 400 ng mL^{-1} for ICP-AES analysis.

4.2 *Method 04.13-B Atomic Absorption Spectrophotometry Methods, US EPA Method 7000A from Document SW846:*

4.2.1 Soluble metals are readily determined by atomic absorption spectrometry. The method is simple, rapid, and applicable to a large number of metals in drinking, surface, and saline waters as well as domestic and industrial wastes. Drinking water free of particulate matter may be analyzed directly, but groundwater, other gaseous samples, environmental pollutant (EP) extracts, industrial waste, soil sludge, sediment, compost, and other solid wastes require digestion prior to analysis for both total and acid leachable metals. Analysis for dissolved elements does not require digestion if the sample was filtered and acidified.

4.2.1.1 Detection limits, sensitivity, and optimum ranges of the metals will vary with sample matrices and models of atomic absorption spectrophotometers. The data shown in Table 04.13-B1 provide some indication of the detection limits obtainable by direct aspiration and by furnace techniques. For clean aqueous samples, the detection limits shown in the table by direct aspiration may be extended downward with scale expansion and upward with a less sensitive wavelength or by rotating the burner head. Detection limits by direct aspiration may also be extended through concentration of the sample and/or through solvent extraction techniques. For certain samples, lower concentrations may also be determined with furnace techniques. The detection limits given in Table 04.13-B1 are dependent upon equipment (e.g., type of spectrophotometer and furnace accessory, the energy source, the degree of electrical expansion of the output signal), and are greatly dependent upon sample matrix. Detection limits should be established empirically for each matrix type analyzed. When using furnace techniques, however, the analyst should be cautioned as to possible chemical reactions occurring at elevated temperatures which may result in either suppression or enhancement of the analysis element. To ensure valid data with furnace techniques, the analyst must examine each matrix for interference and, if detected treat them accordingly, with successive dilution, matrix modification, or method standard additions.

4.2.1.2 Where direct-aspiration atomic absorption spectrophotometry techniques do not provide adequate sensitivity, reference is made to specialized procedures (in addition to the furnace procedure) such as the gaseous-hydride method for arsenic and selenium and the cold-vapor technique for mercury.

Table 04.13-B1 Atomic absorption spectrophotometry concentration ranges.

	Direct Aspiration		*Furnace Procedure* [a, c]
Metal[1]	*Detection Limit mg L^{-1}*	*Sensitivity mg L^{-1}*	*Detection Limit µg L^{-1}*
Aluminum	0.1	1	-.-
Antimony	0.2	0.5	3
Arsenic[b]	0.002	-.-	1
Barium	0.1	0.4	2
Beryllium	0.005	0.025	0.2
Cadmium	0.005	0.025	0.1
Calcium	0.01	0.08	-.-
Chromium	0.05	0.25	1
Cobalt	0.05	0.2	1
Copper	0.02	0.1	1
Iron	0.03	0.12	1
Lead	0.1	0.5	1
Lithium	0.002	0.04	-.-
Magnesium	0.001	0.007	-.-
Manganese	0.01	0.05	0.2
Mercury[d]	0.0002	-.-	-.-
Molybdenum (p)	0.1	0.4	1
Nickel	0.04	0.15	-.-
Osmium	0.03	1	-.-
Potassium	0.01	0.04	-.-
Selenium[b]	0.002	-.-	2
Silver	0.01	0.06	0.2
Sodium	0.002	0.015	-.-
Strontium	0.03	0.15	-.-
Thallium	0.1	0.5	1
Tin	0.8	4	-.-
Vanadium	0.2	0.8	4
Zinc	0.005	0.02	0.05

NOTE 1—(p) means use of pyrolytic graphite furnace.
a—For furnace sensitivity values, consult instrument operating manual.
b—Gaseous hydride method.
c—Listed furnace values are expected when using a 20 µL injection and normal gas flow, except for arsenic and selenium, where gas interrupt is used.
d—Cold vapor technique.

5. Significance and Use

5.1 Method 04.13-A Cold Vapor AAS Technique for Mercury in Compost:

5.1.1 This Cold Vapor AA method is a modification of US EPA Method 7470A and is applicable to US EPA SW846 Method 3051 digest described for compost in TMECC Method 04.12-A.

5.1.2 Organic mercurials and inorganic forms of mercury may also be present in compost. Because organo-mercury compounds will not respond to the cold vapor atomic absorption spectrophotometry or ICP-AES analytical techniques, organic mercury must be broken down and converted to mercuric ions. The dissolution of organic mercury is largely achieved with TMECC Method 04.12-A (US EPA Method 3051 Modified) using concentrated nitric acid and microwave heating.

5.1.3 When compost contains more than 5 mg kg^{-1} of Hg, the ICP-AES determinations may be performed on the US EPA Method 3051 digests. When high accuracy is needed, additional sample preparation is required and the cold vapor AA method (US EPA Method 7470) is recommended. Organic mercury must be completely converted to the mercury ion which can then be reduced to gaseous elemental mercury. Half normal sulfuric acid, potassium permanganate, and potassium persulfate are used in addition to the nitric acid present in the US EPA 3051 digest. Sulfuric acid and potassium permanganate oxidize many of the organic mercury compounds, but potassium persulfate is used to obtain complete oxidation. Specifically, potassium persulfate is needed to oxidize alkyl mercurials, including phenyl mercuric acetate and methyl mercuric chloride.

5.2 *Method 04.13-B Atomic Absorption Spectrophotometry Methods, US EPA Method 7000A from Document SW846:*

5.2.1 Although methods are reported for the analysis of solids by atomic absorption spectrometry, the technique generally is limited to metals in solution or solubilized by some form of sample processing.

5.2.2 Preliminary treatment of wastewater, ground water, EP extracts, and industrial waste is always necessary because of the complexity and variability of sample matrix. Solids, slurries, and suspended material must be subjected to solubilization process before analysis. This process may vary and depends upon the metals determined and the nature of the sample analyzed. Solubilization and digestion procedures are presented in *TMECC Method 04.12 Digest Techniques*.

5.2.3 In direct-aspiration atomic absorption spectrometry, a sample is aspirated and atomized in a flame; and this produces free unexcited ground-state atoms in the flame. A light beam from a hollow cathode lamp or an electrodeless discharge lamp is directed through the flame into a monochromator, and onto a detector that measures the amount of absorbed light. The wavelength is chosen to match the adsorption characteristics of the metal being determined. Because of this, the light energy absorbed by the metal atoms in the flame is a measure of the concentration of that metal in the sample. This principle is the basis of atomic absorption spectrometry.

5.2.4 When using the furnace technique in conjunction with an atomic absorption spectrophotometer, a representative aliquot of a sample is placed in a graphite furnace, evaporated to dryness, charred, and atomized. As a greater percentage of available atoms is vaporized and dissociated for absorption in the tube of the furnace rather than the flame, the use of smaller sample volumes or detection

of lower concentration of elements is possible. The principle of the technique is essentially the same as the direct aspiration atomic absorption, except that a furnace, rather than a flame, is used to atomize the sample. Radiation from a given excited element is passed through the vapor containing ground-state atoms of that element. The intensity of the transmitted radiation decreases in proportion to the amount of the ground-state element in the vapor. The metal atoms to be measured are placed in the beam of radiation by increasing the temperature of the furnace, thereby causing the injected specimen to be volatilized. A monochromator is used to obtain the characteristic radiation from the hollow cathode lamp or electrodeless discharge lamp, and a photosensitive device measures the attenuated transmitted radiation.

6. Interference and Limitations

6.1 *Method 04.13-A Cold Vapor AAS Technique for Mercury in Compost:*

6.1.1 Possible interference from sulfide is eliminated by the addition of potassium permanganate. Concentrations as high as 20 mg L^{-1} of sulfide do not interfere with the recovery of added inorganic mercury from distilled water.

6.1.2 Copper was also found to interfere, but copper concentrations as high as 10 mg L^{-1} had no effect on recovery of mercury from spiked samples.

6.1.3 Water vapor which absorbs ultraviolet light at 253.7 nm is effectively removed with a condensation trap followed by a drying tube.

6.1.4 Chloride interference and ions are removed by the addition of hydroxylamine sulfate just prior to reduction by $SnCl_2$.

6.2 *Method 04.13-B Atomic Absorption Spectrophotometry Methods, US EPA Method 7000A from Document SW846*

6.2.1 The most troublesome type of interference in atomic absorption spectrophotometry is chemical; the metal of interest is in molecular combination with other atoms and the flame is not sufficiently hot to dissociate the molecule. This is the case when Mg is compounded with phosphate or when the dissociated atom is immediately oxidized to a compound that will not dissociate further at the temperature of the flame. The addition of lanthanum will overcome phosphate interference in magnesium, calcium, and barium determinations. Similarly, addition of calcium to mixtures of magnesium and silica will allow for accurate determinations of Mg concentrations.

6.2.2 *Direct Aspiration Procedure:*

6.2.2.1 Complexing agents may also be used to eliminate or reduce atoms or compounds that interfere with the analysis of a specific metal.

6.2.2.2 The presence of high concentrations of dissolved solids in the sample may cause the absorption to increase and results in an overestimation of the concentration of metal in a sample. This is determined by making the measurement with radiation that does not absorb the metal. Samples containing high concentrations of dissolved solids should be cleaned up and the solids removed.

6.2.2.3 Ionization occurs when the flame temperature is sufficiently high to generate the removal of an electron from a metal atom, (i.e., produce cations). This can generally be controlled by addition, to both standard and sample solutions, of an excess (1,000 mg L^{-1}) of an easily ionized element such as Li, Na, K, or Cs.

6.2.2.4 Spectral interference occurs when an absorbing wavelength of an element, present in the sample but not being determined, falls within the width of the absorption line of the element of interest. The concentration of metal will be overestimated. Problems can also occur when the same metal impurity in the lamp cathode or multielement lamp is found in the solution and falls within the bandpass of the slit setting. Narrowing the slit width sometimes reduces this problem..

6.2.2.5 Samples and standards should be monitored for viscosity differences because this may alter their aspiration rate.

6.2.2.6 All metals are not equally stable in the digest, especially if it contains only nitric acid, not nitric acid and hydrochloric acid. The digest should be analyzed as soon as possible, especially if Sn, Sb, Mo, Ba, and Ag are being measured.

6.2.3 *Furnace Procedure*—Oxide formation is greatly reduced with furnace procedures because atomization occurs in an inert atmosphere, but the analyst should be aware that oxides still form. The composition of the sample matrix can also have a major effect on the analysis and must be considered. The serial dilution technique may be used. Samples which exhibit these type of problems should be treated in one or more of the following ways:

6.2.3.1 Successively dilute and re-analyze the samples to eliminate oxide/compound formation.

6.2.3.2 Modify the sample matrix either to remove contaminants and/or stabilize the metal. Examples are the addition of ammonium nitrate to remove alkali chlorides and the addition of ammonium phosphate to react with cadmium. Mixing of hydrogen with the inert purge gas has also been used; hydrogen acts as a reducing agent and aids in molecular dissociation.

6.2.3.3 Analyze the sample by the furnace method while noticing the precautions and limitations of its use:

6.2.3.3.1 Gases generated in the furnace during atomization may have molecular absorption bands that overlap with the analytical wavelength. When this occurs, use either background correction or choose an alternate wavelength. Background correction may also compensate for nonspecific broad-band absorption interference;

6.2.3.3.2 Continuum background correction cannot correct for all types of background interference. When the background interference cannot be compensated for, chemically remove the contaminant or use an alternate form of background correction, e.g., Zeeman background correction;

6.2.3.3.3 Some matrices produce smoke that can interfere with the analysis. Volatilizing at higher temperatures or extending the length of the charring time sometimes reduces this problem. Utilizing an ashing cycle in the presence of air might also be useful, but care must be taken to prevent the loss of metal;

6.2.3.3.4 Samples that contain large amounts of organic materials should be oxidized by conventional acid digestion before being placed in the furnace. In this way, broad-band absorption is minimized;

6.2.3.3.5 Nitric acid is the preferred acid for digestion or solubilization. If another acid in addition to nitric acid is required, a minimum amount should be used. This applies particularly to hydrochloric and, to a lesser extent, to sulfuric and phosphoric acids. The anions of these acids interfere more with the analysis of metals than nitric acid;

6.2.3.3.6 Metals may form carbides within the furnace, (e.g., molybdenum). When carbides form, the metal is released very slowly from the resulting metal carbide. Molybdenum may require 30 s to atomize. Carbide formation is greatly reduced and sensitivity increased with the use of pyrolytically coated graphite;

6.2.3.3.7 Cross-contamination and contamination within the sample is a major sources of error because of the extreme sensitivities achieved with the furnace. The sample preparation work area should be kept scrupulously clean. All glassware should be cleaned in the following sequence: detergent rinsed with tap water, 1:1 nitric acid rinsed with tap water, 1:1 hydrochloric acid rinsed with tap water, and then followed with two final rinses of 17 MΩ·cm water. Pipette tips are a frequent source of contamination and they should be acid soaked with 1:5 nitric acid and rinsed thoroughly with tap band reagent water. Special attention should be given to reagent blanks in both analysis and in the correction of analytical results. Finally, pyrolytic graphite, can become contaminated. As many as five to ten high-temperature burns may be required to clean the tube before use.

7. Quality Control for Atomic Absorption Spectrophotometry Techniques

7.1 All quality control data should be maintained and available for easy reference or inspection.

7.2 A calibration curve must be prepared each day with a minimum of a calibration blank and three standards. After calibration, the calibration curve must be verified by use of at least a calibration blank and a calibration check standard that are made from a reference material or other independent standard material and that fall near the mid-range. The calibration reference standard must be measured within 10% of its true value for the curve to be considered valid.

7.3 If more than 10 samples per day are analyzed, the working standard curve must be verified by measuring a mid-range standard or reference standard after every 10 samples. This sample value must be within approximately 10% of the true value, or the previous ten samples need to be reanalyzed.

7.4 At least one matrix spike and one matrix spike duplicate sample must be included in each analytical batch. A laboratory control sample shall also be processed with each sample batch.

7.5 Where the sample matrix is so complex that viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition (MSA) is recommended.

7.6 *Interference Tests:*

7.6.1 *Dilution Test*—For each analytical batch, select one typical sample for serial dilution to determine if problems exist. The concentration of the metal should be at least 25× the estimated detection limit. Determine the apparent concentration in the undiluted sample. Dilute the sample by a minimum of five-fold (1+4) and reanalyze. If all of the samples in the batch are below 10× the detection limits, perform the spike recovery analysis described below. Agreement within 10% between the concentration for the undiluted sample and five times the concentration of the diluted sample is good, and such samples may be analyzed without the method of standard additions.

7.6.2 *Recovery Test*—If results from the dilution test do not agree, interference originating from the matrix with the may be suspected, and a spiked sample should be analyzed to help confirm the finding from the dilution test. Withdraw another aliquot of the test sample and add a known amount of metal solute to bring the concentration of the metal solute to 2 to 5× the original concentration. If all of the samples in the batch have metal solute concentrations below the detection limit, spike the selected sample at 20× the detection limit. Analyze the spiked sample and

calculate the spike recovery. If the recovery is less than 85% or greater than 115%, the method of standard additions must be used for all samples in the batch.

7.6.3 *Method of Standard Additions*—This technique involves adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the metal solute signal, thus producing a different slope from that of the calibration standards. It will not correct for baseline shift. The method of standard additions must be used for analysis of all EP extracts, on all analyses submitted as part of a delisting petition, and whenever a new sample matrix is being analyzed.

7.6.3.1 The simplest version of this technique is the single-addition method, in which two identical aliquots of the sample solution, each of a volume V_x, are taken. To the first (labeled A) is added a known volume V_S of a standard metal solute of concentration C_S. To the second aliquot (labeled B) is added the same volume V_S of the solvent. The analytical signals of A and B are measured and corrected for non metal solute signals. The unknown sample concentration C_x is calculated:

$$C_x = (S_B \times V_S \times C_S) \div (S_A - S_B) \times V_x \quad \textit{Equation 7.6.3.1}$$

where:

S_A and S_B = analytical signals (corrected for the blank) of solutions A and B, and

V_S and C_S = chosen so that S_A is roughly twice S_B on average, avoiding excess dilution of the sample.

NOTE 2—If a separation or concentration step is used, the additions are best made at the beginning of the entire procedure.

7.6.3.2 Improved results can be obtained by employing a series of standard additions. To equal volumes of the sample are added a series of standard solutions containing different known quantities of the metal solute with all solutions diluted to the same final volume. For example, addition should be prepared so that the resulting concentration is approximately 50% of the expected absorbance from the endogenous metal solute in the sample. Addition 2 and 3 should be prepared so that the concentrations are approximately 100% to 150% of the expected endogenous sample absorbance. The absorbance of each solution is determined and then plotted on the vertical axis of a graph (dependent variable, y), with the concentrations of the known standards plotted on the horizontal axis (independent variable, x). When the resulting line is extrapolated to zero absorbance, the point of interception of the abscissa is the endogenous concentration of the metal solute in the sample. The abscissa on the left of the ordinate is scaled the same as on the right side, but in the opposite direction from the ordinate. Refer to FIG 04.13-1 for an example of a standard addition plot. Linear regression may be used to obtain the intercept concentration.

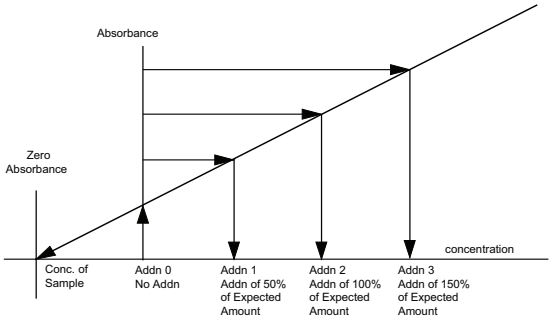


Fig 04.13-1 Conceptual example of a standard addition plot.

7.6.3.3 For the results of this MSA technique to be valid, the following limitations must be taken into consideration:

7.6.3.3.1 The apparent concentrations from the calibration curve must be linear over the concentration range. For the best results, the slope of the MSA plot should be nearly the same as the slope of the standard curve. If the slope is significantly different (> 20%), caution should be exercised.

7.6.3.3.2 There should be no variation as the ratio of metal concentration to sample matrix changes, and the standard addition should respond in a similar manner as the metal solute concentration.

7.6.3.3.3 The measurement must be free of spectral interference and corrected for nonspecific background interference.

8. Sample Handling

8.1 *Method 04.13-A Cold Vapor AAS Technique for Mercury in Compost*—Use compost digest derived from air-dried (36°C), milled, inert-free compost using TMECC Method 04.12-A Microwave Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost.

8.2 *Method 04.13-B Atomic Absorption Spectrophotometry Methods, US EPA Method 7000A from Document SW846*—Use compost digest derived from air-dried (36°C), milled, inert-free compost using TMECC Method 04.12-A Microwave Assisted Nitric Acid Digestion, SW-846 US EPA Method 3051 Modified for Compost, or refer to selected digest method for specific sample handling and preparation procedures.

Test Method: Atomic Absorption Spectrometry. Cold Vapor AAS Technique for Mercury in Compost							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.13-A	04.13-A						04.13-A	04.13-A

04.13-A COLD VAPOR AAS TECHNIQUE FOR MERCURY IN COMPOST

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

9. Apparatus for Method A

9.1 *Atomic Absorption Spectrophotometer*—MercuryMonitor™, (LDC Analytical, Riviera Beach, Florida). This spectrophotometer has (matched) dual path flow cell path lengths of 30 cm.

9.2 *Recorder*—linear multiple range recorder, Instruments Corp.

9.3 *Gas Aspirator Apparatus*—a 150-mm fritted glass filter stick (cat #9436-06, Ace Glass Inc.) for generation and purging of Hg vapor from sample to detector with N_2.

9.4 *Tubing*—various tubing may be used interchangeably to carry N_2 and Hg vapor between cold vapor AA components. Minimized dead space in the tubing is advantageous, but larger tubing is sometimes dictated by the tubing connections for some components. Tygon tubing (o.d. 7 mm) compatible with the flow meter is reduced to o.d. 5 mm to fit the nipple on the reference flow cell. Further reduction to o.d. 1.5 mm polyethylene tubing compatible to a three way switch, allows the carrier gas to bypass the reaction vessel between sample runs to re-establish baseline. Larger polyethylene tubing (i.e., o.d. ≥ 3 mm) helps control pressure build up as mercury vapor to conducted from the reaction vessel to the flow cell, via the condensation trap and drying tube. Short lengths of silicon tubing (o.d. 8 mm) facilitate the connection of larger components, i.e. the condensation trap, drying tube, and flow meter.

9.5 *Reaction Vessel*—where inorganic mercury is reduced to volatile Hg with $SnCl_2$. It consists of a 50-mL polyethylene centrifuge tube (Evergreen) fitted with a 3-hole stopper to accommodate the in and out flow of N_2 carrier gas and the addition of a standard amount (1 mL) of $SnCl_2$. The N_2 is bubbled into the reaction vessel with a 150-mm sintered glass filter stick (Ace Glass Inc. #9436-06)

9.6 *Water Condensation Trap*—a 30-mL vacuum type distillation trap with a 24/40 fitting (Ace Glass Inc.) immersed in a ice water bath.

9.6.1 *Drying Tube*—a glass tube, 15 × 1.9 cm (6 × $^3/_4$ in.), containing ~ 2 g of magnesium perchlorate is located between the ice water bath and the second dual path flow (sample) cell. Magnesium perchlorate is replaced before each daily analysis.

9.6.2 *Water Trap*—Silicon tubing (i.d. 6 mm) is used to connect the water trap and drying tube, then down-sized to a ~ 2 cm length of tygon tubing (o.d 5 mm) to return the carrier gas to the second flow cell.

9.6.3 The two flow cell chambers separate the 253.7 nm radiation from a common source lamp into two beams that ultimately impinge on a solid state dual photocell. One element of the photocell senses the 253.7 nm radiation through the Hg vapor (sample) flow cell, and the other element senses 253.7 nm radiation transmitted through the reference flow cell. The difference between the two transmitted beams is the additional absorption (or reduced transmission) caused by the presence of the sample cell.

9.7 *Centrifuge Tubes*—disposable 50-mL plastic (Evergreen free standing).

9.8 *Volumetric Pipettes*—1-mL, 5-mL (TD).

9.9 *Volumetric Flask*—100-mL.

9.10 *Beaker*—500-mL.

9.11 *Three-way valve*—Teflon™.

10. Reagents and Materials for Method A

10.1 *Reagent Water*—All references to water is this method refer to (Tube I) reagent water unless otherwise specified. Reagent grade water will be of at least 17 MΩ·cm quality.

1.2 *Sulfuric Acid Solution (0.5N)*—Add 7 mL of concentrated Baker instra-analyzed (or equivalent reagent grade) sulfuric acid to 500-mL volumetric flask. Bring to volume with water and thoroughly mix.

10.3 *Potassium Permanganate Solution (5% w v^{-1})*—In a 100-mL volumetric flask, dissolve 5 g of reagent grade $KMnO_4$ in 20 mL of water, bring to volume and thoroughly mix.

10.4 *Potassium Persulfate Solution (*5% w v^{-1}*)*—In a 100-mL volumetric flask, dissolve 5 g of reagent grade

potassium persulfate ($K_2S_2O_8$), bring to volume and thoroughly mix.

10.5 *Stannous Chloride Solution (10 %, w v^{-1})*—Dissolve 50 g of reagent grade $SnCl_2 \cdot H_2O$ in 50 mL of concentrated Baker instra-analyzed (or equivalent reagent grade) concentrated HCl in a 500-mL volumetric flask. Bring to volume with water and mix thoroughly.

10.6 *Hydroxylamine Sulfate Solution (*12%)—Dissolve 12 g of reagent grade NaCl and 12 g of reagent grade hydroxylamine sulfate in water in a 100-mL volumetric flask. Bring to volume with water and mix thoroughly.

10.7 *Cylinder of Nitrogen Gas.*

10.8 *Nitric Acid*—concentrated (Baker instra-analyzed or equivalent reagent grade).

10.8.1 *Nitric Acid (2.5 % v v^{-1})*—Add 25 mL of the above concentrated reagent grade nitric acid to a 1-L volumetric flask containing approximately 750 mL of water. Add more water to make a final volume of 1 L.

10.9 *Working Mercury Standards*—Prepare standards in 2.5 % nitric acid.

Note A1—Water samples run by US EPA 7471A are commonly preserved with 1% nitric acid. Such samples and matching Hg standards in 1% nitric acid require additional concentrated nitric acid (2.5 %) for oxidation of Hg by US EPA 7471A. For this compost procedure it is easier to prepare standards directly in 2.5% nitric acid.

10.9.1 *Stock solution containing 10 µg mL^{-1} mercury*—Using pipette, add 1 mL of the commercial 1000 µg mL^{-1} stock solution (Plasmachem or equivalent) to a 100-mL volumetric flask containing 2.5 mL concentrated nitric acid (Baker instra-analyzed or equivalent reagent grade) and approximately 50 mL of deionized water. Bring up to volume (100 mL) with water.

10.9.2 *Stock solution containing 100 ng mL^{-1} mercury*—Using pipette, add 1 mL of 10 µg mL^{-1} mercury to a 100-mL volumetric flask. Bring up to volume with 2.5 % nitric acid solution.

10.9.3 *Stock solution containing 10 ng mL^{-1} mercury*—Using pipette, add 10 mL of 100 ng mL^{-1} mercury to a 100-mL volumetric flask. Bring up to volume with 2.5 % nitric acid solution. This standard stock solution used for water analysis is not used for compost that contains much more mercury.

10.9.4 *Standards Solutions*—Add the described volumes of the *100 ng mL^{-1} mercury* stock solution (Table 04.13-A1) to five separate 100-mL volumetric flasks. Bring up to volume with 2.5 % nitric acid and mix thoroughly. A wider range of standards e.g. from 1 to 100 ng Hg mL may be necessary when SRMs or compost samples contain unusually low or high concentrations of Hg.

10.9.4.1 Although the addition of 0.05 g of $K_2Cr_2O_7$ to each 100 mL standard will increase their use for a month, it is recommended that mercury standards be prepared daily.

Table 04.13-A1 Standard Hg solutions.

Volume of Stock Solution (100ng Hg mL^{-1})	*Concentration (ng Hg mL^{-1})*
1.0 mL	1.0
2.0 mL	2.0
3.0 mL	3.0
4.0 mL	4.0
5.0 mL	5.0

Note A2—Standards containing $K_2Cr_2O_7$ (w v^{-1}) should be discarded after one week of storage.

11. Procedure for Method A

11.1 *Digestion Steps for Water Standards:*

11.1.1 Using a volumetric pipette add 1 mL of each working standard to 50-mL centrifuge tube with 4 mL of 2.5 % nitric acid.

11.1.2 Add 0.25 mL of the sulfuric acid (0.5 *N*) solution to each working standard with volumetric dispenser and mix by gently shaking the centrifuge tubes.

11.1.3 Add 0.75 mL of the 5% (w v^{-1}) potassium permanganate solution to each working standard with a volumetric dispenser.

11.1.4 Add 0.5 mL of the 4% (w v^{-1}) potassium persulfate solution to each working standard with a volumetric micropipette. Tightly cap each centrifuge tube and mix by gently shaking each flask.

11.1.5 Place centrifuge tubes in holding trays and submerge in a 95°C water bath for 2 h. Cool to room temperature (~27°C). The standards are now fully oxidized and ready for analysis

11.2 *Digestion Steps for Compost.* This digest is performed following US EPA Method 3051A (Method 04.12-A). This method is adapted for compost; 0.5 g of compost is digested with 10 mL of concentrated nitric acid diluted to 40 mL.

11.2.1 Transfer 0.5 mL of the 2.5% nitric acid digest solution into a mercury free 50 mL centrifuge tube.

11.2.2 Add 4.5 mL of deionized water (17 MΩ·cm pure) to obtain the required 2.5% nitric acid concentration.

11.2.3 Add 0.25 mL of 0.5 *N* sulfuric acid (0.5 *N*), 0.75 mL potassium permanganate (5% w v^{-1}), additional permanganate may be required for some wastes. Shake and add enough permanganate so that the purple color persists for at least 15 min.

11.2.4 Add 0.5 mL of potassium persulfate (4% w v^{-1}), and cap the centrifuge tube tightly.

11.2.5 Place the capped centrifuge tube in a 95°C water bath, and digest for 2 h as required for US EPA Method 7470A. After digestion, remove the sample tubes and equilibrate at room temperature (~27°C). The standards are now fully oxidized and ready for analysis.

11.3 *Cold Vapor AA Analysis:*

11.3.1 Start the MercuryMonitor™ and Recorder. The front panel meter on the MercuryMonitor™ should be in the 0.1 to 0.6 range. An instrument warm up period of at least half an hour is required.

11.3.2 Add fresh anhydrous magnesium perchlorate to the drying tube and connect it between the water trap and input for the reference cell, using glass fiber to contain the drying reagent. Also add fresh ice to a 500-mL beaker surrounding the water condensation trap and a small amount of water to insure good contact.

11.3.3 Turn on the N_2 gas cylinder. Set the regulator to about 6.5 PSI to create and maintain a gas flow of 250 mL min^{-1}. Set chart speed to 4 in. h^{-1}.

11.3.4 Excess permanganate must be neutralized before the sample is analyzed by cold vapor AA for mercury. Add hydroxylamine-NaCl solution drop wise and shake vigorously until the solution clears. (Usually at least 3 drops are required).

11.3.5 Remove the cap from the first working standard and connect to the Cold Vapor Assembly by use of the 3-hole stopper. In this way each centrifuge tube becomes a reaction vessel in which the now fully oxidized Hg is reduced to its volatile (Hg^0) form.

11.3.6 Turn the 3-way injection switch down causing the carrier N_2 gas to bypass the reaction vessel, establish the base line by adjusting the recorder.

11.3.7 Open pinch clamp and add 0.25 mL of the $SnCl_2$ using the volumetric dispenser. Close pinch clamp. Move 3-way injection switch upward to cause gas to flow through the reaction vessel and fritted glass bubbler.

11.3.8 Each working standard will produce a peak on the recorder.

11.3.9 Wait until the recording pen returns to about 95 % of the baseline level before the LDC switch is thrown to bypass the reaction vessel. The pen should return to the baseline by the time the next standard is prepared.

11.3.10 Repeat this procedure for all standards.

11.3.11 Repeat this procedure for the compost samples as follows:

1.1.1.1 Just prior to the generation of cold-vapor and the determination of Hg, add three drops of the hydroxylamine sulfate solution (12%) to reduce the excess permanganate. Re-cap the tube and shake the digest vigorously until it becomes clear. Some samples may require an additional drop of hydroxylamine sulfate to make the digest clear.

12. Calculations and Corrections for Method A

12.1 Determine standard graph. Plot absorbance (mm, y axis) of standards against total amount of Hg in flask digest (ηg, x-axis) and determine the slope M (mm mL μg^{-1}) and intercept B (mm).

12.2 Determine the total Hg (ηg) within the digest aliquot:

$$T = (A - B) \div M \qquad \textit{Equation 12.2}$$

where:

T = total mass of mercury in 0.5 mL 3051 digest aliquot, ηg,
A = sample response, mm,
B = intercept of standard curve, mm, and
M = slope of standard curve, mm mL μg^{-1}.

12.3 Determine the total mass of Hg (ηg) in the 3051 digest:

$$D = (T \div 0.5) \times 40 \qquad \textit{Equation 12.3}$$

where:

D = total mass of mercury in the 3051 sample digest, ηg,
T = total mass of mercury in the 0.5 mL 3051 digest aliquot in ηg,
0.5 = volume of 3051 compost digest in reaction vessel, mL, and
40 = volume of digest, mL.

12.4 Determine the mass of Hg (mg) per kg of oven dried compost:

$$E = (D \div W \times 1000) \qquad \textit{Equation 12.4}$$

where:

A = cold vapor AA response of sample, mm,
B = intercept of standard curve, mm,
T = total mass ÷ of mercury in the 0.5 mL 3051 digest aliquot in ηg ,
M = slope of standard curve, mm mL μg^{-1},
D = total mass of mercury in the 3051 sample digest, ηg ,
0.5 = volume of 3051 compost digest in reaction vessel, mL,
E = mass of mercury in digest per kg of oven dried compost, mg kg^{-1},
40 = volume of digest, mL,
1000 = conversion from g to kg, and
W = weight of the sample on an oven dried basis, g.

Test Method: Atomic Absorption. Atomic Absorption Spectrophotometry							**Units:** mg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.13-B	04.13-B						04.13-B	04.13-B

04.13-B ATOMIC ABSORPTION SPECTROPHOTOMETRY

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

ADAPTED FROM—SW-846 US EPA METHOD 7000A. It is a copy of the AA method presented in SW846, formatted to conform to the style of TMECC.

13. Apparatus for Method B

13.1 *Atomic Absorption Spectrophotometer*—single- or dual-channel, single- or double-beam instrument equipped with a grating monochromator, photomultiplier detector, adjustable slits, a wavelength range of 190 nm to 800 nm, and provisions for interfacing with graphical display.

13.2 *Burner*—use the burner recommended by the particular instrument manufacturer. For certain elements the nitrous oxide burner is required.

13.3 *Hollow Cathode Lamps*—single-element lamps are preferred, but multi-element lamps may be used. Electrodeless discharge lamps may also be used when available. Other types of lamps meeting the performance criteria of this method may be used.

13.4 *Graphite Furnace*—any furnace device capable of reaching the specified temperatures is satisfactory.

13.5 *Graphical Display and Recorder*—a recorder is recommended for furnace work. Keeping a permanent record of conditions helps when troubleshooting problems with the analysis such as drift, incomplete atomization, losses during charring, changes in sensitivity, peak shape, etc.

13.6 *Pipettes*—µL, with disposable tips. Sizes can range from 5 to 100 µL as required. Pipette tips should be checked for contamination prior to use. The accuracy of automatic pipettes must be verified daily. Class A pipettes can be used for the measurement of volumes larger than 1 mL.

13.7 *Pressure-Reducing Valves*—the supplies of fuel and oxidant should be maintained at pressures somewhat higher than the controlled operating pressure of the instrument with the use of suitable valves.

1.8 *Glassware*—glassware, polypropylene, or Teflon containers, including sample bottles, flasks and pipettes, should be washed with the following sequence: detergent rinsed with tap water, 1:1 nitric acid rinsed with tap water, 1:1 hydrochloric acid rinsed with tap water, and then followed with a final rinse of 17 MΩ·cm water. Chromic acid should not be used as a cleaning agent for glassware if chromium is measured. If it can be shown that certain steps in the cleaning procedure are not required for routine samples, those steps may be eliminated from the procedure. Documentation with an analytical quality control program with spiked samples and reagent blanks is necessary before such steps can be eliminated.

14. Reagents and Materials for Method B

14.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. All reagents should be analyzed to provide proof that all constituents are below the MDLs.

14.2 *Reagent Water*—all references to water in this method refer to reagent water unless otherwise specified. Reagent grade water will be of at least 17 MΩ·cm quality.

14.3 *Nitric Acid—(concentrated), HNO_3*—use a spectrograde acid certified for AA use. Prepare a 1:1 dilution with water by adding the concentrated acid to an equal volume of water. If the reagent blank is less than the IDL, the acid may be used.

14.4 *Hydrochloric Acid (1:1), HCl*—use a spectrograde acid certified for AA use. Prepare a 1:1 dilution with water by adding the concentrated acid to an equal volume of water. If the reagent blank is less than the IDL, the acid may be used.

14.5 *Fuel and Oxidant*—high purity acetylene is generally acceptable. Air may be supplied from a compressed air line, a laboratory compressor, or a cylinder of compressed air and should be clean and dry. Nitrous oxide is also required for certain measurements. Standard, commercially available argon and nitrogen are required for furnace work.

14.6 *Stock Standard Metal Solutions*—stock standard solutions are prepared from high purity metals, oxides, or nonhygroscopic salts using water and redistilled

nitric or hydrochloric acids (see individual methods for specific instructions). Sulfuric or phosphoric acids should be avoided as they produce an adverse effect on many elements. The stock solutions are prepared at concentrations of 1,000 mg of the metal per liter. Commercially available standard solutions may also be used. Where the sample viscosity, surface tension, and components cannot be accurately matched with standards, the method of standard addition (MSA) may be used.

14.7 *Calibration Standards*—for those instruments, which do not report in concentration units, a calibration curve is prepared to cover the appropriate concentration range. Usually, this means the preparation of standards with an absorbance of 0.0-0.7. Calibration standards are prepared by diluting the stock metal solutions at the time of analysis. For best results, calibration standards should be prepared fresh each time a batch of samples is analyzed. Prepare a blank and at least three calibration standards in graduated amounts in the appropriate range of the linear part of the curve. The calibration standards should be prepared with the same type of acid or combination of acids and at the same concentration as the samples. Beginning with the blank and working toward the highest standard, aspirate the solutions and record the readings. Repeat the operation to secure a reliable average reading for each solution. Calibration standards for furnace procedures should be prepared as described on the individual sheets for that metal. Calibration curves are always required.

15. Procedures for Method B

15.1 *Digest Sample*—Preliminary treatment of compost is always necessary because of the complexity and variability of sample matrices. This process may vary because of the metals to be determined and the nature of the sample being analyzed. Digestion procedures are presented in the TMECC 04.12 Digest Techniques.

15.2 *Direct Aspiration (Flame) Procedure:*

15.2.1 Differences among the various makes and models of atomic absorption spectrophotometers make it impracticable to outline detailed instructions that are applicable to every instrument. The analyst should follow the manufacturer's operating instructions for a particular instrument. In general, after choosing the proper lamp for the analysis, allow the lamp to warm up for a minimum of 15 min, unless operated in a double-beam mode. During this period, align the instrument, position the monochromator at the correct wavelength, select the proper monochromator slit width, and adjust the current according to the manufacturer's recommendation. Subsequently, light the flame and regulate the flow of fuel and oxidant. Adjust the burner and nebulizer flow rate for maximum percent absorption and stability. Balance the photometer. Run a series of standards of the element under analysis. Construct a calibration curve by plotting the concentration of the standards against absorbance. If using a direct reading instrument set the curve corrector to record the proper concentration. Aspirate the samples and determine the concentrations either directly or from the calibration curve. Standards must be run each time a sample or series of samples is run.

15.3 *Furnace Procedure:*

15.3.1 Furnace devices (i.e., flameless atomization) are useful means of extending detection limits. The analyst should follow the instructions provided by the manufacturer of the particular instrument.

15.3.2 Background correction is important with flameless atomization, especially below 350 nm. Certain samples, when atomized, may absorb or scatter light from the lamp. This can be caused by the presence of gaseous molecular species, salt particles, or smoke in the sample beam. If no correction is made, sample absorbance will be overestimated, and the analytical result overestimated. Zeeman background correction is effective in overcoming composition or structured background interference. It is particularly useful when analyzing for As in the presence of Al and when analyzing for Se in the presence of Fe.

15.3.3 Memory effects (i.e., cross contamination) occur when the metal is not totally volatilized during atomization. This condition depends upon several factors: volatility of the element; the use of pyrolytic graphite; the rate of atomization and furnace design. This situation is detected through blank burns. The tube should be cleaned by operating the furnace at full power for the required time period, and as needed, at regular intervals during the series of determinations.

15.3.4 Inject a measured µL aliquot of sample into the furnace and atomize. If the concentration found is greater than the highest standard, the sample should be diluted in the same acid matrix and reanalyzed. The use of multiple injections can improve accuracy and help detect furnace and pipette errors.

15.3.5 To verify the absence of interference, follow the serial dilution procedure. (Refer to 7.6.1 *Dilution Test*).

15.3.6 A check standard should be run after approximately every 10 injections of sample. Standards are run in part to monitor the life and performance of the graphite tube. Lack of reproducibility or significant change in the signal for the standard indicates that the tube should be replaced. Tube life depends upon sample matrix and atomization temperature. A conservative estimate is that it should

last at least 50 firings. A pyrolytic coating will extend the estimated life by a factor of three.

16. Calculation for Method B

16.1 Calculate concentration in sample, $\mu g\ g^{-1}$

$$M = [A \times (V - (1 - TS) \times F)] \div O \qquad \textit{Equation 16.2}$$

where:

M = µg metal per g sample, $\mu g\ g^{-1}$,
A = metal in processed sample from calibration curve, µg,
V = final volume of the processed sample, mL,
TS = total solids ratio determined on parallel sample aliquot, unitless,
F = mass of the AA processed sample aliquot at as-received moisture, g, and
O = oven dry weight of sample, g.

16.2 Report all concentrations in consistent units corrected to dw basis, determined on a parallel sample aliquot dried at 70±5°C.

04.13 METHODS SUMMARY

17. Report

17.1 *Methods 04.13-A and 04.13-B*—Report measurements to 0.01 mg kg^{-1}. Report digest method. Report all concentrations in consistent units corrected to dw basis determined with a parallel sample aliquot dried at 70±5°C.

18. Precision and Bias

18.1 *Method 04.13-A Cold Vapor AAS Technique for Mercury in Compost, Modified US EPA Methods 7470A*—The precision of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

Table 04.13-A2 Intra-sample precision from three sites and two sample dates for mercury.

Median	*Std Dev*	*% CV*	*Number of Samples*
2.6	0.23	8.9	10
2.2	0.13	5.7	9
7.7	0.64	8.3	10
6.1	0.39	6.3	10
6.5	0.60	9.0	10
7.1	0.58	8.1	10

18.2 *Method 04.13-B Atomic Absorption Spectrophotometry Methods, US EPA Method 7000A from Document SW846*—The precision and bias of this test are not determined. Data are being sought for use in developing a precision and bias statement.

19. Keywords

19.1 atomic absorption; heavy metals; metal; mercury; cold vapor

Test Method: Inductively Coupled Plasma Analysis. One Method.							**Units:** $\mu g\ mL^{-1}$	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.14-A	04.14-A						04.14-A	04.14-A

04.14 INDUCTIVELY COUPLED PLASMA ANALYSIS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

CONTRIBUTED BY—Robert O. Miller

1. Scope

1.1 This section covers elemental determinations using Inductively Coupled Plasma techniques.

1.1.1 *Method 04.14-A Inductively Coupled Plasma-Atomic Emission Spectroscopy, US EPA Method 6010A*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. References

2.1 *TMECC:*

Method 03.09 Total Solids and Moisture.

Method 04.13 Digestion Techniques

2.2 *Other Sources:*

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3. Terminology

3.1 *element, n*—A substance composed of atoms having an identical number of protons in each nucleus. Elements cannot be reduced to simpler substances by normal chemical means.

3.2 *spectroscopy, n*—Study of spectra, especially experimental observation of optical spectra.

3.3 *standard, n*—Serving as or conforming to a standard of measurement or value. Sample often referred to a standard reference sample or check of known physical, chemical or biological characteristics used to monitor analytical bias or accuracy of a physical, chemical or biological determination.

4. Summary of Test Methods

4.1 *ICP-AES and ICP-MS.* ICP is generally superior in accuracy, precision, detection limit, freedom from interferences, and dynamic range than other analytical instrumentation. The use of automatic samplers, large computers, and appropriate software facilitates accurate and rapid analysis. One can analyze a solution for many elements in 1 min (ICP-AES); therefore, large volumes of data can be generated very fast. Isaac and Johnson (1982) indicate that with ICP-AES one technician can do the same work that formerly required four technicians. Thought should be given to the handling and processing of the data. Interfacing the instrument with larger computers for data handling and analysis is a must if one contemplates obtaining large volumes of data. It is recommended that users of ICP-AES and ICP-MS subscribe to the ICP-Information Newsletter (Department of Chemistry, University of Massachusetts, Amherst) and other newsletters available from the manufacturer of their unit so that they can be kept abreast of new developments in ICP-AES and ICP-MS. Journals such as Applied Spectroscopy, Analytical Chemistry, Analytical Chimica Acta, and others referred to in this chapter are also good sources of information. Additional sources of information have been described in the text.

4.2 *ICP-AES and ICP-MS Instrumentation*—The ICP is produced by passing initially ionized Ar gas through a quartz torch located inside a Cu coil connected to a

radio frequency (RF) generator. The RF generator provides up to 3 kW forward power (in most commercial units) at a frequency of 27.1 MHz. The high-frequency currents flowing in the Cu coil generate oscillating magnetic fields whose lines of force are axially oriented inside the quartz tube and follow elliptical closed paths outside the coil (Fassel, 1977; Fassel and Kniseley, 1974). Electrons and ions passing through the oscillating electromagnetic field flow at high acceleration rates in closed annular paths inside the quartz tube space. The induced magnetic fields direction and strength vary with time resulting in electron acceleration on each half cycle. Collisions between accelerated electrons and ions, and ensuing unionized Ar gas cause further ionization.

4.2.1 The collisions cause ohmic heating and, when measured spectroscopically, give thermal temperatures ranging from 6,000 to 10,000 °K (Fassel, 1977). However, with the advent of the ICP-MS, it is evident that the true thermal temperature of the plasma is much lower than this. For example, the Perkin Elmer SCIEX 500 that has been in the DANR Analytical Lab for over a year, has run for hours with the "6000 °K" region of the plasma striking the copper interface plate with no melting or etching of the copper metal surface. In addition, several ICP-MS laboratories use copper as the sampler cone metal (Hieftje and Vickers, 1989; Houk, 1986). Copper appears to give satisfactory results in this role unless sulfuric acid is present in the test solutions and the sampler cone aperture is relatively small (i.e. *cf.* 0.4 mm); in which case, rapid erosion has been observed (Munro et al., 1986). Copper metal melts at 1356°K and boils at 2840°K (Weast and Astle, 1979).

4.2.2 The quartz torch has three concentric channels. The outer channel conducts Ar gas at about 15 L min^{-1} to 17 L min^{-1} to the plasma to sustain the plasma and to isolate the quartz tube from high temperatures. The innermost channel is for introduction of sample into the plasma. The middle channel conducts the auxiliary Ar gas at about 1 L^{-1} min^{-1} and is used in ICP-AES only when starting the plasma or for organic samples, and is routinely used for all types of samples for ICP-MS. The ICP has an annular, or donut, shape when it is viewed from above. The hole has a lower temperature than the donut body and offers less resistance to the sample injection. The sample is injected into the plasma by using Ar carrier gas at a rate of about 1 L^{-1} min^{-1} for ICP-AES work. For ICP-MS work the aerosol flow is approximately 1.5 L min^{-1}.

4.3 *Properties of ICP*—The ICP generated, as discussed above, has unique physical properties that make it an excellent source for vaporization/atomization/ionization/excitation of elements.

4.3.1 *Method 04.14-A Inductively Coupled Plasma-Atomic Emission Spectroscopy, US EPA Method 6010A*—the aerosol droplets containing the analyte are desolvated, the analyte salts/oxides are vaporized, and the analyte atomized at the high temperature region of the plasma in the vicinity of the Cu coil. An initial radiation zone (IRZ) has been defined by Koirtyohann et al. (1980) as the zone that begins in the sample aerosol channel inside the load coil for ICP-AES. The IRZ extends upward to one or two mm above the load coil, taking on the appearance of an amber "bullet" during nebulization of many sample types related to agriculture. This is due to emission from CaO molecules on the surface of the "bullet", the color changing to a deep blue or purple further downstream as emission from calcium atoms and ions dominates. The blue/purple region is termed the normal analytical zone (NAZ), and is the region in which the analyte emission is observed by the spectrometer. Color photographs illustrating the appearance of the IRZ and NAZ while nebulizing an elevated concentration of yttrium into an ICP have recently been published for ICP-AES (Winge et al., 1988), and more clearly define these critical regions. The NAZ is 15-mm to 20-mm above the coil, or about 14-mm to 19-mm above the tip of the IRZ, in an environment relatively low in background emission. The background consists of Ar lines and some weak band emission from OH, NO, and CN molecules present in the plasma (Ward, 1978a). By the time the decomposition products of the sample reach the NAZ, they have had a residence time of about 2 msec at spectroscopically measured temperatures ranging from about 8,000 to 5,000 °K (Fassel, 1977). The residence time and temperature experienced by samples introduced into the plasmas are about twice as large as those in the hottest flames, e.g., N_2O-C_2H_2. The high temperature and residence time combination, at the sample aerosol flow rates typically used in ICP-AES, lead to complete sample vaporization and atomization in contrast to flames that require releasing agents for refractory compounds (Larson et al., 1975). Once the free compounds, atoms, and ions are formed in ICP-AES, they are in a chemically inert environment in contrast to highly reactive combustion flame environments. Ionization interferences are generally negligible in an ICP-AES experiment. Self-absorption (a phenomenon responsible for the flattening of the standard curve at high analyte concentrations) is practically absent, which leads to a wide linear dynamic analytical range of 3 to 5 decades. No sampling or skimmer cones, and lense stack or quadrupole rods are used in the ICP-AES, and therefore, contamination from ablative processes off of them, e.g. secondary ion sputtering, is absent.

4.3.2 *ICP-MS*—the vaporization and atomization begin at approximately the same location relative to the

load coil as do these processes in the ICP-AES, in a relatively hot region of the plasma in the vicinity of the Cu coil. However, the flow rates of sample and/or auxiliary argon are increased for ICP-MS to obtain an analytically useful population of ions (Winge, et al., 1991), while keeping the sampling cone a safe distance from the load Cu coil to prevent arcing between the cone and the load cu coil. The IRZ extends well beyond the downstream side of the load cu coil. The water droplets produced in a conventional concentric nebulizer, although apparently extremely few in number compared to the total number of aerosol droplets produced, can survive the rigorous desolvation/atomization conditions generated by the ICP (Winge et al., 1991). Although the downstream side of the load coil-to-IRZ tip distance varies from one lab to another, it is generally between 10 and 20 mm for ICP-MS. Unlike ICP-AES, this leaves much of the analyte vaporization and atomization to be done in regions beyond the hottest parts of the ICP in the ICP-MS case. The sampling cone orifice defines the NAZ in the ICP-MS, and is another 2- to 10-mm downstream from the tip of the IRZ. In the DANR Analytical Lab, the IRZ extends approximately 19 mm downstream from the spectrometer side of the load coil and the sampler cone orifice is positioned another 3 mm downstream from the IRZ tip; which results in placement of the NAZ a total of 22 mm from the nearest surface of the load coil. Most of the particle beam is sucked through the sampling cone into the intermediate vacuum region of a differentially pumped aperture approximately two to three mm from the tip of the bullet. The tip of a second cone, called the skimmer, is immersed in what is termed a barrel shock (Gray, 1989) that results from supersonic expansion of the plasma gas as it passes from atmospheric pressure through the sampling cone orifice into a vacuum of about 1 torr. The kinetic temperature of the gaseous particles at the tip of the skimmer cone has been measured to be 2200 $^{\circ}$K (Lim et al., 1989; Winge, et al., 1991). Although the position of the sampler with respect to the extended IRZ of the ICP results in a maximum rate of ions per second at the detector, it also is sampling aerosol that has undergone solute vaporization and atomization reactions outside the hottest regions of the ICP. This is thought to contribute to the appearance of more molecular ions in the mass spectra and higher susceptibility to non-spectroscopic matrix effects than if the aerosol flow rate and/or auxiliary argon flow rate could be slowed down enough to put the IRZ back to within one or two mm of the downstream side of the load coil. However, this is not possible because of the arcing that occurs between the load coil and the metallic sampling cone in instances in which the cone is placed too close spatially to the load coil. We have been unsuccessful at locating descriptions of ICP-MS experiments designed to reduce molecular ion formation in the mass spectrum using a sampler constructed of a sampling cone that does not conduct electricity. Among the possibilities for non-conducting materials are high tech ceramics that could withstand prolonged exposure to the highest temperature regions of the ICP. These include AlN, SiC, Al_2O_3, or zirconia ceramics[1]. The sampler could be placed so that the NAZ is in a region closer to local thermodynamic equilibrium (LTE) with respect to maximized ion populations while the analyte solute vaporization and atomization is allowed to proceed in the hottest parts of the plasma.

4.4 *Normal Analytical Zone*—In general, the normal analytical zone (NAZ) is much closer to the tip of the IRZ in ICP-MS (2 to 10 mm) than the NAZ is to the tip of the IRZ in ICP-AES (14 to 19 mm). The closer proximity used for the ICP-MS measurements increases the concentration of ions to a level at which they are analytically useful (Winge et al.., 1991). Ideally, ions should be extracted from a region that approximates local thermal equilibrium (LTE). Ion temperatures are sufficient to support high ion populations at this proximity to the IRZ tip. The requirement for high ion density at a distance well downstream from maximum gas and excitation temperatures promotes formation of metal oxide ions and non-spectroscopic concomitant suppression effects that are observed in the ICP-MS. Modifications involving the usual sample introduction techniques have been found to significantly reduce these problems.

5. Significance and Use

5.1 *Method 04.14-A Inductively Coupled Plasma-Atomic Emission Spectroscopy, US EPA Method 6010A*—New developments in ICP-AES include: interfacing ICP spectrometers with flow injection analyzers for automatic dilution, calibration, separation, concentration, standard additions and other operations (Greenfield, 1983; LaFerniere, et al., 1985); interfacing ICP-AES with liquid chromatographs for concentration and speciation of elements (Roychowdhury and Koropchack, 1990); high salt nebulizers to prevent clogging of nebulizers (Legere and Burgener, 1985); successful use of concentration and reduction of spectral interferences techniques such as chelation/ solvent extraction (Huang and Wai, 1986; Bradford and Bakhtar, 1991); use of computer programs such as orthogonal polynomials (Hassan and Loux, 1989), simplex optimization (Belchamber et al., 1986), and that recommended by Taylor and Schutyser, 1986, for optimization of spectrometer operating conditions and automatic correction for spectral interferences.

1 Coors Ceramics, 9th and Ford Street, Golden, CO

6. Interference and Limitations

6.1 Elemental coverage and detection limits under relatively ideal conditions are excellent. There are problem areas in ICP-MS that must be investigated (Hiefje, 1992). Most of the following problems have been overcome or circumvented to meet analytical needs in selected instances. The statements that follow are generally valid for a generic, normal resolution, i.e. peak widths between 0.5 and 1.0 dalton, and normal aqueous aerosol generation ICP-MS:

6.1.1 The accuracy and precision of ICP-MS data are typically three times less as compared to ICP-AES. However, for concentrations determined from isotope dilution/ratio measurements, precision and accuracy is somewhat better than concentrations determined by ICP-AES (Gregoire, 1989; Dolan et al., 1990).

6.1.2 Isobaric overlaps (spectral interferences) occur with some regularity for elements between approximately 28 to 80 daltons, and do occur throughout the mass range. They are a result of a common unit mass shared by more than one element, doubly charged ions overlapping a singly charged isotope with half the unit mass of the doubly charged species (Vaughan and Horlick, 1986), elemental oxide, elemental hydride, and/or elemental hydroxide ions overlapping isotopes of other elements (Vaughan and Horlick, 1986; Munro et al., 1986; Gray, 1986), and background spectral problems (Vaughan and Horlick, 1986; Gray, 1986; Tan and Horlick, 1986). The isobaric interferences involving oxygen can be eliminated using techniques such as electrothermal vaporization (ETV), atomization or Laser ablation sample aerosol production (Gregoire, 1989).

6.1.3 Ion response is significantly suppressed by concomitant concentrations. The threshold concomitant values are low compared to emission suppressions noted for ICP-AES. Non-spectroscopic interferences result from excessive dissolved solids in the test solutions. For a number of reasons, the analyte ion arrival rate at the detector, i.e. analyte response, is suppressed under these circumstances (Beauchemin et al., 1987; Olivares and Houk, 1986; Douglas and Kerr, 1988; Gregoire, 1987a, 1987b; Hieftje, 1992). Although at the DANR Analytical Lab, the onset of suppression is usually observed in the neighborhood of 100 to 500 mg L^{-1}, Gregoire indicates somewhat higher levels using the same instrument model/manufacturer (Perkin-Elmer SCIEX 250, Gregoire, 1989).

6.1.4 The ICP generated in argon with normal aqueous solution nebulization may be unable to produce measurable amounts of positive ions for some analytes that could be of interest, (e.g. F, Cl, and/or S). However, the halogens can be determined in the negative ion mode (Hieftje et al., 1988; Chisum, 1992), while sulfur can be detected if the water is removed from the sample prior to nebulization. Water vapor can be removed from the sample aerosol using a cooled spray chamber (Hutton and Eaton, 1987). Water can be completely separated from the sulfur using an electrothermal atomizer (Gregoire, 1989) or partially removed using nebulization - desolvation equipment (Veillon and Margoshes, 1968).

6.1.5 The cost of instrumentation, operation, and maintenance for ICP-MS are generally higher than those for ICP-AES, leading to higher cost per analyte concentration determination. The cost per analyte concentration determination for an off-the-shelf ICP-MS is about two and one-half times that of a state-of-the-art automated sequential scanning ICP-AES instrument using the same depreciation schedule for each instrument. Gregoire (1989) points out, however, that the relative cost of analysis using ICP-MS is low relative to other methods capable of producing data on individual isotopes. Similarly, the sample throughput is about a factor of five greater for ICP-MS than obtainable by other isotope methods.

7. Sample Handling and Preparation

7.1 Refer to the following TMECC digest procedures for descriptions of sample handling and preparation.

7.1.1 Method 04.12-A Microwave-Assisted Nitric Acid Digestion.

7.1.2 Method 04.12-B Nitric Acid Digestion, US EPA 3050A Modified.

7.1.3 Method 04.12-C Dry Ash Sample Digestion for Plant Nutrients.

7.1.4 Method 04.12-D Water-Soluble Elements.

7.1.5 Method 04.12-E Aqua-Regia Procedure.

7.2 Wet digestion is performed on an air-dried sample (36°C) by methods listed above. Inductively coupled plasma (ICP) analysis is applied to digests.

7.2.1 Volatile loss of As and Hg will occur at higher temperatures, (e.g. > 36°C). Mercury determination may be performed on a subsample of the EPA-3051 digest with the cold vapor atomic absorption method.

7.3 The measured data are adjusted and reported on an oven dried basis. Moisture and total solids content is determined on a parallel aliquot of the air-dried sample by oven-drying at 70±5°C until weight change due to moisture loss diminishes to nil.

Test Method: Inductively Coupled Plasma Spectroscopy						**Units:** mg mL^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.14-A	04.14-A						04.14-A	04.14-A

04.14-A INDUCTIVELY COUPLED PLASMA-ATOMIC EMISSION SPECTROSCOPY, US EPA METHOD 6010A

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

COMMENT—This method description is for reference only and was provided by the Research Analytical Laboratory, Department of Soil, Water, and Climate, University of Minnesota, St. Paul 55108. Refer to specific manuals for methods and procedures appropriate for other ICP instruments and laboratory operation.

8. Apparatus for Method A

8.1 *Inductively Coupled Atomic Emission*—1 m, vacuum, simultaneous reading multi-element spectrometer equipped with twenty seven element capability and background correction. Fison Instruments, ARL Model 3560 or other equivalent ICP-AES.

Table 04.14-A2 ICP wavelength table for use with the Dry Ash Acid digestion procedure. Wavelengths (nm) for each element are listed.

Element	*Wavelength (nm)*	*Element*	*Wavelength (nm)*
Ca	317.93	Cu	324.75
Al	308.20	Cr	308.22
Fe	259.94 & 233.28	B	182.59
Na	589.59	As	189.04
Mg	279.08	Cd	226.50
K	766.49	Mo	202.03
P	213.62	Ni	231.60
Mn	257.60	Zn	213.86

1.2 *Random Access Sample Changer*—Gilson model 222, with five 44-position sample trays.

9. Reagents and Materials for Method A

9.1 *Nitric Acid*—70%, J.T. Baker Intra-Analyzed™ grade for trace element analyses.

9.2 *Reagent Water*—Deionized minimum resistivity of 17 MΩ·cm (minimum standard).

1.3 *ICP Aspirated Rinse Solution*—10% "Instra-Analyzed" hydrochloric acid.

1.4 *Calibration Standards for Two-Point Calibration*—Refer to Table 04.14-A2.

Table 04.14-A3 Standard 1, zero concentration: 25% dilution of 4 *M* stock J.T. Baker Instra-Analyzed™ nitric acid.

Elements	*Standard 1[1] concentration (mg mL^{-1})*	*mL stock[2] 10,000 mg mL^{-1}*	*%[3]*
Ca	600	30	4.8
Al	300	15	2.4
Fe	300	15	2.4
Na	120	6	0.96
Mg	100	5	0.8
P	50	2.5	0.4
K	100	5	0.8
Mn	20	1	1600 *(mg kg^{-1})*
Zn	20	1	1600 *(mg kg^{-1})*

1—concentration in solution of calibration Standard 2;
2—mL of stock solution diluted to 500 mL for Standard 2;
3—equivalent concentration in dry compost.

Table 04.14-A4 Standard 2, multi-element, in 4 M stock J.T. Baker Instra-Analyzed™ nitric acid.

Elements	*Standard 1[1] concentration (mg mL^{-1})*	*mL stock[2] 1,000 mg mL^{-1}*	*mg $kg^{-1, 3}$*
Pb	12	6	960
Cu	10	5	800
Cr	2	1	160
B	2	1	160
As	1	0.5	80
Cd	1	0.5	80
Mo	1	0.5	80
Ni	1	0.5	80

1—concentration in solution of calibration Standard 2;
2—mL of stock solution diluted to 500 mL for Standard 2;
3—equivalent concentration in dry compost.

10. Procedure for Method A

10.1 *Sample Digest*—Refer to *TMECC Method 04.12 Digest Techniques* for sample digestion procedures. Transfer approximately a 10 mL aliquot of the diluted digest to 17 mL polyethylene disposable tubes. Cap the tubes firmly.

10.1.1 Place sample tubes in Gilson-222 sample trays and enter sample identifiers and other test parameters into Set-up file for automatic analysis under computer control.

10.2 *Interference and Corrections*—Create an analysis program to contain the following test parameters:

10.2.1 Previously determined inter-element spectral corrections for the elements showing interfering spectra for the elements listed above.

Note A1—Linear correction for the Fe spectra within the Cd spectrum region is not adequate and will result in a serious Cd error at Fe concentrations that are 1000× greater than Cd. A quadratic equation is employed to correct for this interference for samples very high in iron (Table 04.14-A.5).

10.2.2 Activate background correction mode for predetermined peak offset background readings for Mo, As, Cd, Ni and Pb. These elements were found to have an elevated background in a typical MSW compost digest.

10.2.3 Read time: three separate 10 sec readings per sample.

10.2.4 *Within-run quality control settings*—Set normalization mode to read std-2 after every ten samples. Set "Normalization" limit for std-2 at 5% for one or more elements.

10.3 *ICP-AES Calibration Procedure:*

10.3.1 After a 30 min warm-up period of aspirating a 10% HCl rinse solution, calibrate the zero concentration point (Std-1), followed by calibration of the high standard (Std-2).

10.3.2 Confirm the calibration by analyzing the above two calibration standards as if they are "sample" solutions. If the measured concentrations are not within 5% of set values, recalibrate.

10.3.3 Proceed with the analysis of samples, blanks and reference samples in the Automated Mode.

10.4 *Analysis:*

10.4.1 Within-run automatic calibration control—After every ten samples, Std-2 is analyzed. If any of the calibrated elements exceed 5% of their known value, the calibration curve is "normalized" on Std-1 and Std-2. If any element in the std-2 set exceeds 10%, the previous set of ten samples is to be re-analyzed after re-calibration.

10.4.2 If the concentration for any of the calibrated elements exceed the known linear range of the spectrometer, the sample is to be diluted and rerun.

10.5 *Quality Control:*

10.5.1 Use of high purity acids and deionized water. See reagents section.

10.5.2 Calibration solutions. Use only spectroscopic grade commercial single and multi-element stock standard solutions. Compare each new set of working standards against the previous set.

10.5.3 Replication of samples and inclusion of reference materials is performed at the digestion phase of the analysis.

10.5.3.1 Included with each batch of 20 samples, is a minimum of one "In-house" MSW-compost check, and one "outside" reference sample of either EPA or the Certified European BCR (Community Bureau of Reference) Industrial sewage Sludge.

10.5.3.2 Spike one or more samples within each batch of 20 samples with a stock solution (multi-element spectrochemical grade) at the digestion phase.

10.5.3.3 Replicate one sample in every ten samples at the digestion phase.

04.14 METHODS SUMMARY

11. Report

11.1 Report determination method and digest method.

11.2 Report concentration of elements relative to the compost sample on an oven dried (70±5°C).

11.3 *Significance*—±0.1% of dilution concentration.

12. Precision and Bias

12.1 *Method 04.14-A Inductively Coupled Plasma-Atomic Emission Spectroscopy US EPA Method 6010A*—The precision of this method was evaluated by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN.

12.1.1 *Precision*—Precision for Elemental Analysis of MSW Compost Standard Reference Sample used in MN-OEA CUP Project (See Table 04.14-A5). All measurements were made by ICP-AES, except for Hg. Hg is determined by Cold-Vapor AAS, refer to TMECC Methods 04.06-Hg and 04.13-A. Digestions were performed by US EPA SW-846 Method 3051 (modified for compost). Data reported in mg kg^{-1} dw unless noted as % dw. The standard reference sample was prepared from MSW compost as follows: material was air-dried at 36°C for two weeks, passed through a 4-mm sieve, milled to a fine powder with a Stein mill equipped with a carbide blade, oven-dried at 68°C for 2 d, mixed in a sample tumbler for 2 d, split into two 3 L aliquots, and stored in two separate sealed neoprene bottles at room temperature (~25°C).

Table 04.14-A5 Precision of heavy metals determinations with standard reference sample over a two year period.

Element	*Max* *mg kg^{-1}*	*Min* *mg kg^{-1}*	*Median* *mg kg^{-1}*	*Mean* *mg kg^{-1}*	*Std Dev* *mg kg^{-1}*	*%CV*	*Number of Samples*
As	17.4	11.2	14.2	14.2	1.1	8	34
Cd	18.40	8.30	9.50	9.99	1.84	18	34
Cr	121.8	58.0	78.9	79.8	11.6	14	34
Cu	341	244	317	314	17	5	34
Pb	784	240	328	343	87	25	34
Hg	11.30	7.90	10.30	10.08	0.85	8	34
Mo	8.93	4.44	7.02	6.79	0.87	13	34
Ni	83.8	43.8	61.8	62.4	7.0	11	34
Se	.	.	.	.	.	.	0
Zn	1797	1185	1661	1652	116	7	34

ADAPTED FROM—Research Analytical Laboratory, Department of Soil, Climate and Water, University of Minnesota, St. Paul, MN 55108.

Table 04.14-A6 Precision of plant nutrient determinations with standard reference sample over a two year period. Values are reported on a dw basis in mg kg^{-1} except where otherwise noted.

Element	*Max*	*Min*	*Median*	*Mean*	*Std Dev*	*%CV*	*Number of Samples*
B	162	114	150	149	8	5	34
Ca %	3.95	2.88	3.84	3.77	0.21	6	34
Cu	341	244	317	314	17	5	34
Fe %	2.42	1.67	2.30	2.26	0.14	6	34
K %	0.649	0.451	0.621	0.612	0.038	6	34
Mg %	0.490	0.337	0.460	0.451	0.031	7	34
Mn	1569	851	1185	1193	103	9	34
Mo	8.93	4.44	7.02	6.79	0.87	13	34
Na %	0.935	0.685	0.911	0.894	0.048	5	34
P %	0.361	0.268	0.331	0.329	0.017	5	34
S %	0.908	0.313	0.869	0.827	0.134	16	34
Zn	1797	1185	1661	1652	116	7	34
Al %	1.87	1.11	1.44	1.43	0.18	13	34

ADAPTED FROM—Research Analytical Laboratory, Department of Soil, Climate and Water, University of Minnesota, St. Paul, MN 55108.

13. Keywords

13.1 spectroscopy; ICP; elemental analysis

Test Method: Soluble Salts. One Method. Electrical Conductivity.						**Units:** dS m^{-1} (mMhos cm^{-1})		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
04.10	04.10							04.10

04.15 SOLUBLE SALTS

REFER TO METHOD 04.10 ELECTRICAL CONDUCTIVITY FOR PROTOCOLS TO DETERMINE SOLUBLE SALTS CONTENT.

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Significance

1.1 Soluble salt concentration is the concentration of soluble ions in a solution, and is determined by measuring the electrical conductivity of the solution. Electrical conductivity varies both with the number and type of ions contained in the solution, and may indicate possible phytotoxicity. Soluble salts in compost dictate its end use. Each user group (e.g., vegetable growers, nursery industry, etc.), has its own set of salinity (soluble salts) standards for specific plants or crops. Refer to the Indicator Ratios section, Method 05.02-E Agricultural Index, for a proposed method designed to manage the relative salt content of feedstock blends.

1.2 This test covers the determination of electrical conductivity of compost.

1.2.1 *Method 04.10-A 1:5 Slurry Method, Mass Basis*.

Test Method: Biodegradable Volatile Solids. Discussion Only						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	05.01	05.01		05.01		05.01		05.01

05.01 BIODEGRADABLE VOLATILE SOLIDS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section refers to the measurement of ash and volatile solids content for compost feedstocks, in-process material and compost products.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 03.02-B Milled Material Ignited at 550°C with Inerts Removal.

Method 03.09-A Total Solids and Moisture.

Method 05.07-A LOI Organic Matter.

2.2 *Other References:*

Cohen, I.R. 1973. Laboratory Procedure for the Preparation of Solid Waste Related Materials for Analysis. *In* Methods of Solid Waste Testing, EPA-6700-73-01. US EPA, Cincinnati, OH 45268.

Method for the Evaluation of Water and Wastewater, EPA 0600/4-79-020, US EPA Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

Standard Methods for the Examination of Water and Wastewater. 1992. Part 2000, Physical and Aggregate Properties. Method 2540 E. Fixed and Volatile Solids Ignited at 500°C.

US EPA Method 600/4-79-020 adapted by physical removal of volatile solids that are not readily biodegradable.

3. Terminology

3.1 *ash, n*—The inorganic matter, or mineral residue of total solids that remains when a compost or feedstock is combusted at 550°C in the presence of excess air; *fixed solids*, % g g^{-1}.

3.2 *biodegradable volatile solids, n*—organic matter, % g g^{-1}.

3.3 *fixed solids, n*—The inorganic matter, ash, or mineral residue of total solids that remains when a compost or feedstock is combusted at 550°C in the presence of excess air; *ash*, % g g^{-1}.

3.4 *moisture content, n*—The liquid fraction (percentage) of a compost or feedstock that evaporates at 70±5°C, % g.g^{-1}.

3.5 *organic matter, n (OM)*—The sum of solids in compost that contain organic carbon; the total organic components in compost including undecayed plant and animal tissues, their partial decomposition products, and the compost biomass exclusive of living macrofauna and macroflora. Determination is made by combusting a sample at 550°C for two hours (Method 05.07-A LOI Organic Matter).

3.6 *total solids, n*—The solid fraction of compost or feedstock that does not evaporate at 70±5°C; this consists of fixed solids, organic matter or biodegradable volatile solids, inorganic carbon, and volatile solids that are not readily biodegradable, % g g^{-1}.

3.7 *volatile solids, n*—The sum of biodegradable materials, non-biodegradable materials, and biodegradable materials that do not degrade during the retention time allowed for composting, but do volatilize to carbon dioxide and other gasses when a compost or feedstock is combusted at 550°C in the presence of excess air, % g g^{-1}.

4. Summary of Relevant Test Methods

4.1 *Method 05.07-A Loss-On-Ignition Organic Matter Method (LOI)*—Organic matter content of a compost sample is determined by igniting an oven-dried sample in a muffle furnace at 550°C. The organic material fraction is volatilized and the mineral fraction

is retained as ash. Percent organic matter content is determined by calculating the difference in mass before and after ignition relative to the bulk oven-dried sample.

4.1.1 The LOI method is a direct determination of compost organic matter. The method is rapid, easy and accurate for properly prepared samples. The compost method is based upon methods developed for use with peat and organic soils.

4.1.2 This test method is based upon similar methods provided in ASTM and AOAC: Test Method C from ASTM D 2974 - 87 (Reapproved 1995). Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils; Test Method 967.05 (final action 1967) from AOAC Official Methods of Analysis Organic Matter in Peat (1990).

4.2 *Method 03.02-A Unmilled Material Ignited at 550°C, Inerts Not Removed*—Quick Test to determine moisture and total solids content at 70±5°C and total ash and volatile solids content by combustion at 550°C in the presence of excess air. Determinations are reported on an oven-dried basis of unsieved, as-received composting feedstock, in-process material or compost product sample.

4.2.1 This test is recommended for samples where no consideration need be given to inert materials and for biodegradable materials that do not degrade during the retention time allowed for composting.

4.3 *Method 03.02-B Milled Material Ignited at 550°C, Inerts Removed*—Analytical test to determine total ash and biodegradable volatile solids contents by combustion at 550°C in the presence of excess air. Determinations are reported on an oven-dried basis from an air-dried (36°C), sieved and milled sample that does not contain non-biodegradable materials or biodegradable materials that do not readily humify.

4.3.1 This test method provides an estimate of biodegradable volatile solids. Inerts and biodegradable materials that do not degrade during the retention time for composting are removed prior to performing this test.

4.4 *Method 03.02-C. Unmilled Material Ignited at 550°C, Inerts Removed*—Analytical test to determine moisture and total solids content at 70±5°C, and total ash and volatile solids content by combustion at 550°C in the presence of excess air. Determinations are reported on an oven-dried basis of a sieved, as-received finished or in-process compost, or feedstock sample.

4.4.1 The test employs a calculated weighting method to compensate for inert materials and for biodegradable materials that do not degrade during the retention time allowed for composing.

Test Method: Indicator Ratios. Seven Methods						**Units:** Unitless		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.02-A		05.02-A				05.02-A
		05.02-B		05.02-B				05.02-B
		05.02-C		05.02-C				05.02-C
		05.02-D		05.02-D				05.02-D
							05.02-E	
05.02-F	05.02-F					05.02-F		05.02-F
						05.02-G		05.02-G

05.02 INDICATOR RATIOS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the calculation of the following indicator ratios:

1.1.1 *Method 05.02-A Carbon to Nitrogen Ratio*.

1.1.2 *Method 05.02-B Carbon to Phosphorus Ratio.*

1.1.3 *Method 05.02-C Ammonium to Nitrate Ratio.*

1.1.4 *Method 05.02-D Carbon to Sulfur Ratio.*

1.1.5 *Method 05.02-E Cadmium to Zinc Ratio.*

1.1.6 *Method 05.02-F Agricultural Index (AgIndex).*

1.1.7 *Method 05.02-G CCQC Maturity Index.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 04.01 Organic Carbon.

Method 04.02-B Nitrate Nitrogen Determination.

Method 04.02-C Ammonium Nitrogen Determination.

Method 04.02-D Total Nitrogen.

Method 04.03-A Total Phosphorus.

Method 04.04-A Total Potassium.

Method 04.05-S Sulfur.

Method 04.05-Na Sodium.

Method 04.05-Fe Iron.

Method 04.06-Cd Cadmium.

Method 04.06-Zn Zinc.

Method 05.08 Respirometry.

Method 05.09-A Organic Matter Reduction.

Method 05.10 Volatile Fatty Acids.

2.2 *Other References:*

Bray, B.J., R.H. Dowdy, R.D. Goodrich, and D.E. Pamp 1985. Trace metal accumulations in tissues of goats fed silage produced on sewage sludge-amended soil. J. Environ. Qual. 14:114-118.

Fukushima, M., A. Ishizaki, M. Sakamoto, and E. Kobayashi. 1973. Cadmium concentration in rice eaten by farmers in the Jinzu River basin. Japan J. Hyg. 28:406-415.

Kienholz, E.W., G.M. Ward, D.E. Johnson, J. Baxter, G. Braude, and G. Stern 1979. Metropolitan Denver sewage sludge fed to feedlot steers. J. Anim. Sci. 48:735-741.

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Mathur, S.P., G. Owen, H. Dinel, M. Schnitzer. 1993. Determination of compost biomaturity. I. Literature review. Biol. Agric. Hort. 10:65-85.

McKenna, I.M., R.L. Chaney, S.H. Tao, R.M. Leach, Jr. and F.M. Williams. 1992. Interactions of plant zinc and plant species on the bioavailability of plant cadmium to Japanese quail fed lettuce and spinach. Environ. Res. 57:73-87.

Methods of Soil Analysis. Part 3-Chemical Methods. Ed: D.L. Sparks. Number 5 in the Soil Science Society of America Book Series. Madison, Wisconsin 1996.

Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc.

Reeves, P.G. and R.L. Chaney. 2001. Mineral nutrient status of female rats affects the absorption and organ distribution of cadmium from sunflower kernels (*Helianthus annuus L.*). Environ. Res. *In Press*.

3. Terminology

3.1 *cadmium, n*—(symbol Cd) A soft, bluish-white metallic element occurring primarily in zinc, copper, and lead ores, that is easily cut with a knife and is used in low-friction, fatigue-resistant alloys, solders, dental amalgams, nickel-cadmium storage batteries, nuclear reactor shields, and in rustproof electroplating. Atomic number 48; atomic weight 112.40; melting point 320.9°C; boiling point 765°C; specific gravity 8.65; valence 2.

3.2 *carbon, n*—(symbol C) a naturally abundant nonmetallic element that occurs in many inorganic and in all organic compounds, exists freely as graphite and diamond and as a constituent of coal, limestone, and petroleum, and is capable of chemical self-bonding to form an enormous number of chemically, biologically, and commercially important molecules. Atomic number 6; atomic weight 12.01115; sublimation point above 3,500°C; boiling point 4,827°C; specific gravity of amorphous carbon 1.8 to 2.1, of diamond 3.15 to 3.53, of graphite 1.9 to 2.3; valence 2, 3, 4.

3.3 *iron, n*—(symbol Fe) A silvery-white, lustrous, malleable, ductile, magnetic metallic element occurring abundantly in combined forms, notably in hematite, limonite, magnetite, and taconite, and used alloyed in a wide range of important structural materials. Atomic number 26; atomic weight 55.847; melting point 1,535°C; boiling point 2,750°C; specific gravity 7.874 (at 20°C); valence 2, 3, 4, 6.

3.4 *nitrogen, n*—(symbol N) a nonmetallic element that constitutes nearly four fifths of the air by volume, occurring as a colorless, odorless, almost inert diatomic gas, N_2, Nitrogen (N) is a component of various minerals of all proteins and used in a wide variety of important manufacturing processes, including ammonia, nitric acid, explosives, and fertilizers. Atomic number 7; atomic weight 14.0067; melting point -209.86°C; boiling point -195.8°C; valence 3, 5.

3.4.1 *nitrate nitrogen, n*—(symbol NO_3-N) negatively charged ion comprised of nitrogen and oxygen; nitrate is an inorganic, water soluble and mobile form of nitrogen; because of its negative charge, it is not strongly held by soil particles (also negative) and can be leached away. The chemical formula for nitrate nitrogen is NO_3^-N.

3.4.2 *ammonium nitrogen, n*—(symbol NH_4-N) the univalent chemical ion NH_4^+, derived from ammonia, whose compounds chemically resemble the alkali metals; ammonium is readily converted to and from ammonia depending upon conditions in the compost pile. The chemical formula of ammonium is NH_4^+.

3.5 *phosphorus, n*—(symbol P) A highly reactive, poisonous, nonmetallic element occurring naturally in phosphates, especially apatite, and existing in three allotropic forms, white (or sometimes yellow), red, and black. An essential constituent of protoplasm, a primary plant nutrient, used in safety matches, pyrotechnics, incendiary shells, and fertilizers and to protect metal surfaces from corrosion. Atomic number 15; atomic weight 30.9738; melting point (white) 44.1°C; boiling point 280°C; specific gravity (white) 1.82; valence 3, 5.

3.6 *sulfur, n*—(symbol S) a pale yellow nonmetallic element occurring widely in nature in several free and combined allotropic forms; a secondary plant nutrient. It is used in black gunpowder, rubber vulcanization, the manufacture of insecticides and pharmaceuticals, and in the preparation of sulfur fertilizers and other compounds such as hydrogen sulfide and sulfuric acid. Atomic number 16; atomic weight 32.064; melting point (rhombic) 112.8°C; (monoclinic) 119.0°C; boiling point 444.6°C; specific gravity (rhombic) 2.07; (monoclinic) 1.957; valence 2, 4, 6.

3.7 *fertilizer, n*—Any of a large number of natural and synthetic materials, including manure and nitrogen, phosphorus, and potassium compounds, spread on or worked into soil to increase its capacity to support plant growth.

3.8 *chloride, n*—(symbol XCl_{2+n}) A chlorine-containing compound formed by the reaction of chlorine (Cl_2) with one of many different elements, both metals and nonmetals. Chlorides formed through reactions with chlorine have high oxidation numbers, (e.g., iron(III) chloride ($FeCl_3$), tin(IV) chloride ($SnCl_4$), or antimony(V) chloride ($SbCl_5$)). Chlorine is relatively inert toward carbon, nitrogen, and oxygen.

3.9 *potassium, n*—(symbol K) A soft, silver-white, highly reactive metallic element that occurs in nature only as a compound with other elements . It is obtained by electrolysis of its common hydroxide (KOH) and found in, or converted to, a wide variety of salts. It is used especially in fertilizers and soaps. Atomic number 19; atomic weight 39.102; melting point 63.65°C; boiling point 774°C; specific gravity 0.862; valence 1.

3.10 *sodium, n*—(symbol Na) A soft, light, extremely malleable silver-white metallic element that reacts EXPLOSIVELY with water (although less so than K), is naturally abundant in combined forms, WHICH ARE

NOT EXPLOSIVELY REACTIVE WITH WATER, especially in common salt (NaCl), and is used in the production of a wide variety of industrially important compounds. Atomic number 11; atomic weight 22.99; melting point 97.8°C; boiling point 892°C; specific gravity 0.971; valence 1.

3.11 *zinc, n*—(symbol Zn) A bluish-white, lustrous metallic element that is brittle at room temperature but malleable with heating. It is used to form a wide variety of alloys including brass, bronze, various solders, and nickel silver, in galvanizing iron and other metals, for electric fuses, anodes, and meter cases, and in roofing, gutters, and various household objects. Atomic number 30; atomic weight 65.37; melting point 419.4°C; boiling point 907°C; specific gravity 7.133 (25°C); valence 2.

4. Summary of Test Methods

4.1 Refer to specific elements for recommended digestion and determination method.

4.2 *Method 05.02-A Carbon to Nitrogen Ratio*—Refer to Method 04.01 (Organic Carbon) and Method 04.02-D (Total Nitrogen).

4.3 *Method 05.02-B Carbon to Phosphorus Ratio*—Refer to Method 04.01 (Organic Carbon) and Method 04.03-A (Phosphorus).

4.4 *Method 05.02-C Ammonium to Nitrate Ratio*—Refer to Method 04.02-C (Ammonium) and Method 04.02-B (Nitrate).

4.5 *Method 05.02-D Carbon to Sulfur Ratio*—Refer to and Method 04.01 (Organic Carbon) and Method 04.05-S (Sulfur).

4.6 *Method 05.02-E Cadmium to Zinc Ratio*—Refer to and Method 04.06-Cd (Cadmium) and Method 04.06-Zn (Zinc).

4.7 *Method 05.02-F Agricultural Inde*x—The *AgIndex* is the ratio of macro nutrient content (Total N + P_2O_5 + K_2O) divided by salts content (Na + Cl) in compost or soils. This ratio is used to determine the acceptability of compost feedstocks and the application rate for finished compost in terms of sodium and/or chloride. The *AgIndex* of the finished product can be used to predict whether sodium or chloride toxicity will occur or whether sodium or chloride will accumulate with repeated compost applications.

4.7.1 *Formula Variable*s—Total N, P, K, Cl and Na. Refer to specific tests for extraction techniques and determination procedures for each element.

4.7.1.1 Determine the mixing ratio of feedstocks to estimate the *AgIndex* of a finished compost product.

4.7.2 *The AgIndex Scale*—values below two are rated as poor and indicate that the compost application rate is limited by sodium and/or chloride. Values over ten are rated as excellent and indicate that the compost application rate may be based on plant/soil nutrient requirements. Values from two through ten indicate that the compost application rate formulas incorporate sodium and/or chloride concentrations of the receiving soil.

4.8 *Method 05.02-G CCQC Maturity Index*—Refer to and Method 05.02-A C:N Ratio and the two methods selected from Table 05.02-G2, Group A and Group B Parameters.

4.8.1 The maturity index considers three characteristics of a product: C:N ratio; stability (microbial activity by respirometry), and potential phytotoxicity (bioassay tests and chemical analyses). The first step excludes materials with a high propensity to immobilize nitrogen (high C:N ratio). Compost-like material with a C:N ratio equal to or greater than 25:1 is categorized as immature, whereas compost with a C:N ratio less than 25:1 is further evaluated. The second step excludes material that is still undergoing active microbial decomposition, (e.g., samples that contain adequate levels of carbon to sustain aerobic microbial activity), and a stability rating assigned: very stable; stable; or unstable. Compost rated as unstable is classed as immature, and compost with the stability rating of very stable or stable is further evaluated. The third step screens for significant levels of phytotoxic compounds, and considers chemical maturity parameters. A compost is assigned a maturity indicator rating of immature, mature or very mature. The outcome from steps two and three are contrasted in a two-way decision matrix and assigned a final maturity rating.

5. Significance and Use

5.1 *Method 05.02-A Carbon to Nitrogen Ratio*—The carbon to nitrogen ratio (C:N) is an indication of nitrogen availability for the process of biological degradation. The C:N ratio is the ratio of total organic carbon to total nitrogen. Total organic carbon is the carbon fraction of organic matter (or biodegradable volatile solids). Total nitrogen includes organic nitrogen plus inorganic nitrogen. The inorganic nitrogen fraction is dominated by ammonia nitrogen NH_4^+–N and nitrate nitrogen NO_3^-–N.

5.1.1 Complex forms of carbon in the biomass are oxidized to form less complex forms during decomposition. Bonds between carbon-containing molecules are broken down and the chemical energy stored in the bonds between carbon atoms results in the conversion of chemical energy to heat. As more bonds

are broken, more heat is released. Temperature rise also indicates the rate of biological activity.

5.1.2 For each mole of oxygen consumed by aerobic organisms, one mole of carbon dioxide is produced. The carbon that is not respired combines with nitrogen and other elements within cells of aerobic organisms to synthesize nitrogenous compounds, i. e., protein, nucleic acids, etc., for protoplasm formation. When carbon and oxygen are depleted, microorganisms eliminate the excess nitrogen as ammonia. Living organisms use about thirty parts carbon for each part nitrogen, so the theoretically optimal carbon to nitrogen ratio for a compostable feedstock is thirty to one (30:1). This ratio, and optimized aeration (oxygen) and moisture are essential for rapid, controlled biological degradation.

5.2 *Method 05.02-B Carbon to Phosphorus Ratio*—A C:P ratio indicates the relative content of carbon to phosphorus. Phosphorus sources include polynucleotides (AMP, ADP, ATP, NAD, NADP, FAD, FMN, coenzyme-A, and sugar nucleotides such as ADP-glucose and GDP-glucose) and nucleic acids (RNA, DNA) which are composed of nucleosides.

5.2.1 An initial C:P ratio of 100:1 to 140:1 should be established along with an initial C:N ratio of 30:1. For example, composting feedstocks high in cellulose and lignified materials often have a low phosphorus content and require not only additional nitrogen to maintain the composting process, but additional phosphorus to sustain microbial activity.

5.3 *Method 05.02-C Ammonium to Nitrate Ratio*—Ammonium to nitrate ratio (NH_4–N:NO_3–N) is a valid indication of compost maturity when the sum of their concentrations is greater than 75 mg kg^{-1} dw.

5.3.1 Ammonium nitrogen is formed as a result of volatile fatty acid metabolism (short chain fatty acids). As composting progresses ammonia is oxidized (consumed) to form nitrates as microbial activity converts organic carbon and other compounds into CO_2. The nitrate form of nitrogen is generally present at very low concentrations during the initial phases of the composting process. Consequently, the NH_4–N:NO_3–N ratio may be very high (e.g., greater than 2000:1) during the initial phases of composting, and will drop significantly while composting proceeds and ammonia is converted to nitrate. During later stages of the composting process as maturity increases, nitrate nitrogen can dominate thereby causing the NH_4–N:NO_3–N ratio to fall below 1:1.

5.4 *Method 05.02-D Carbon to Sulfur Ratio*—Information describing the significance and use of a Carbon to Sulfur ratio is being sought to construct a significance and use statement.

5.5 *Method 05.02-E Cadmium to Zinc Ratio*—The Cd:Zn ratio is used as a screening tool to approximate Cd bioavailability.

5.5.1 Assessment of the ultimate bioavailability to humans of soil Cd transferred through foods is very complex. As long as Cd:Zn of crops is near the natural level of less than 0.01, most livestock and wildlife have no increase of Cd in tissues used as food even when crop Cd is increased substantially, i.e., within limits imposed by Zn phytotoxicity and inhibition of Cd transport to grain by Zn. Crop Zn inhibits Cd absorption and/or retention in animal tissues, even liver and kidney (Reeves and Chaney, 2001; McKenna et al., 1994).

5.6 *Method 05.02-F Agricultural Index*—The *AgIndex* is a diagnostic tool used to manage feedstock blends and to optimize the ratio of nutrient to Na and Cl of the finished compost product. Compost feedstock contains sodium and chloride in addition to macro nutrients. The *AgIndex* provides a rating system that separates compost feedstock blends according to their nutrient to [Na + Cl] ratio and predicts this nutrient balance of the finished compost product.

5.6.1 Some industries use high concentrations of sodium and/or chloride in their manufacturing processes and may eventually find there way into compost feedstock blends. Examples include seaweed, ocean fish products, waste water used to wet compost, crops grown on high-salt soils, mericulture products, crops where salts are used as defoliants (e.g., gin trash), pickel waste, cheese, dairy manures, tannery waste, etc. Each of these examples may contain high levels of N, P_2O_5 and K_2O that are inhibited because of correcpondingly high Na and/or Cl concentrations.

5.7 *Method 05.02-G CCQC Maturity Index*—The California Compost Quality Council (CCQC) Maturity Index is a three-step decision tool for classifying composts by their relative level of maturity.

5.7.1 There is widespread acceptance of the need for developing measures of compost maturity and stability as part of a compost quality program. There is a notable lack of consensus about how much emphasis should be placed on measures of maturity vs. stability. The maturity index represents an advance in integrating the measures of maturity and stability.

6. Interference and Limitations

6.1 *Method 05.02-A Carbon to Nitrogen Ratio*—When measuring C:N of either feedstock or finished product, the ratio must be that of % total organic carbon to % total nitrogen, i.e., includes total organic plus inorganic nitrogen.

6.1.1 Total Kjeldahl Nitrogen (TKN) alone is not always an adequate indicator of nitrogen status, although it includes organic nitrogen and ammonia nitrogen, it does not include nitrate nitrogen which may be present at increasing quantities in stable compost.

6.1.2 Refer to Method 04.02 (Nitrogen) and Method 04.01 (Organic Carbon) for specific details.

6.2 *Method 05.02-B Carbon to Phosphorus Ratio*— Refer to Method 04.03 (Phosphorus) and Method 05.01 (Organic Carbon) for specific details.

6.3 *Method 05.02-C Ammonium to Nitrate Ratio*— The NH_4-N:NO_3-N ratio has little value and should not be considered a valid Group B parameter to establish a Compost Maturity Index Rating for composts with very low concentrations of both NH_4–N and NO_3–N (including NO_2–N), i.e., their sum is less than approximately 75 to 100 mg kg^{-1} dw. Refer to Method 05.02-G CCQC Maturity Index for additional maturity indices.

6.4 *Method 05.08-D Carbon to Sulfur Ratio*—Refer to Method 04.05-S. Sulfur and Method 05.01 Organic Carbon for description.

6.5 *Method 05.02-E Cadmium to Zinc Ratio*—

6.6 *Method 05.02-F Agricultural Inde*x—Proper application of the *AgIndex* requires optimum edaphic conditions of target soil and the compost in question, (e.g., optimal compost and soil texture, water holding capacity, porosity, aeration, bulk density, pH, etc.). Factors that commonly limit crop growth after compost application include: 1) high sodium or chloride levels; 2) biologically unstable material (rapid oxygen uptake and carbon dioxide evolution); and 3) the presence of toxins generally associated with anaerobic conditions or immature compost products. The *AgIndex* is used to diminish the probability that sodium and chloride or deficient nutrients become the limiting factor.

6.7 *Method 05.02-G CCQC Maturity Index*— Anticipate continued refinement of the numerical thresholds presented in Tables 05.02-G3 and 05.02-G4.

6.7.1 A Maturity Index should not be the sole indicator for determining compost use. Use instructions should consider multiple compost analytical parameters, (e.g., pH, soluble salts, sieve size, nutrient content, AgIndex, etc.).

7. Sample Handling

7.1 *Method 05.08-A Carbon to Nitrogen Ratio*— Refer to Method 04.02-D Total Nitrogen and Test Method 04.01 Organic Carbon for description.

7.2 *Method 05.08-B Carbon to Phosphorus Ratio*— Refer to Test Method 05.01-A Organic Carbon and Test Method 04.03-A Total Phosphorus and for descriptions.

7.3 *Method 05.08-C Ammonium to Nitrate Ratio*— Refer to Method 04.02-B and Method 04.02-C for descriptions.

7.4 *Method 05.08-D Carbon to Sulfur Ratio*—Refer to Method 04.05-S. Sulfur and Test Method 04.01 Organic Carbon for description.

7.5 *Method 05.02-E Cadmium to Zinc Ratio*—Refer to Test Methods 04.06-Cd and 04.06-Zn for descriptions.

7.6 *Method 05.02-F Agricultural Inde*x—Refer to Method 04.02-D Total Nitrogen, Method 04.03-A Total Phosphorus, Method 04.04 Potassium, and Test Methods 04.05-Na and 04.05-Cl in Secondary and Micronutrients for specific descriptions.

7.7 *Method 05.02-G CCQC Maturity Index*—Sample handling requirements vary with method selected. Refer to cited methods for instruction.

Test Method: Indicator Ratios. Carbon to Nitrogen Ratio							**Units:** unitless	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.02-A		05.02-A				05.02-A

05.02-A CARBON TO NITROGEN RATIO

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Calculations for Method A

8.1 *Calculate C:N Ratio:*

C:N = C ÷ TN *Equation 8.1*

where:

C:N = OC to N ratio, unitless,

C = percent organic carbon, %, and refer to *Test Method 04.01 Organic Carbon*, and

TN = percent total nitrogen, %, refer to *Method 04.02-D Total Nitrogen by Combustion and Reduction.*

9. Interpretation of Results for Method A

9.1 The initial carbon to nitrogen ratio is essential information for feedstock preparation, where the data are used to calculate feedstock blending ratios to obtain an initial C:N ratio in the range of 25-40:1, ideally 30:1 for most feedstock blends.

9.2 Under controlled conditions the C:N ratio in the compost can be an indicator of stability, but to be of value as an indicator, the initial C:N ratio must be at or near the ideal of 30:1, finished compost C:N drops to below 21:1, while phosphorus (P) levels must remain adequate to assure decomposition of any cellulosic material that may be present in the feedstock. (See Fig 05.02-A)

9.3 The C:N ratio in compost is not a stand-alone indicator of stability or maturity, so other indicators must be considered such as respirometry, pH, bulk density, reduction of organic matter, and self-heating.

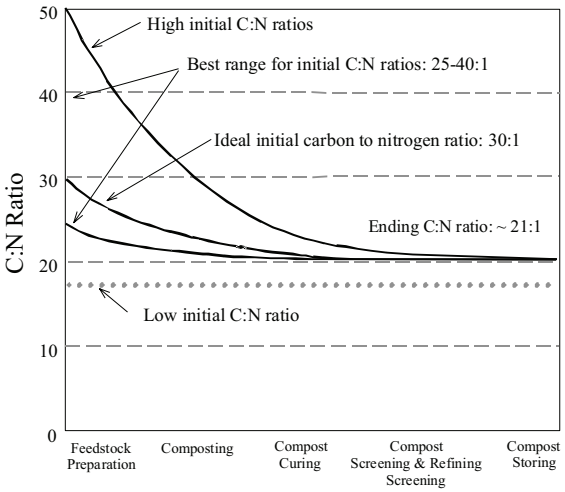

Fig 05.02-A Optimal carbon to nitrogen ratio change for the five steps of the composting process.

Test Method: Indicator Ratios. Carbon to Phosphorus Ratio							**Units:** unitless	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.02-B		05.02-B				05.02-B

05.02-B CARBON TO PHOSPHORUS RATIO

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

10. Calculations for Method B

10.1 *Calculate C:P Ratio:*

$$C{:}P = C \div P \qquad \textit{Equation 10.1}$$

where:

C:P = C to P ratio, unitless,

C = percent organic carbon, %, and refer to *Test Method 04.01-A Organic Carbon*, and

P = concentration of phosphorus, % dw, refer to *Method 04.03-A Total Phosphorus.*

11. Interpretation of Results for Method B

11.1 As composting progresses, microbial activity decreases organic carbon content by converting it and other compounds into CO_2 and H_2O.

11.2 *Research Suggests*—A high initial C:P ratio of greater than 140:1 for composting feedstocks indicates that the phosphorus content is very low and suggests that the bulk of the compost feedstock is high in carbon, such as woody material, paper pulp, refined paper, rayon or other materials high in cellulose and/or lignin. A low C:P ratio causes the composting process to proceed slowly and inefficiently. The rate and efficiency of composting is improved with the addition of phosphorous to reach an innitial C:P ratio of 100:1 to 140:1. Feedstocks know to have a high phosphorus content include household waste, vegetable trimmings, biosolids, yard debris and manures such as poultry litter.

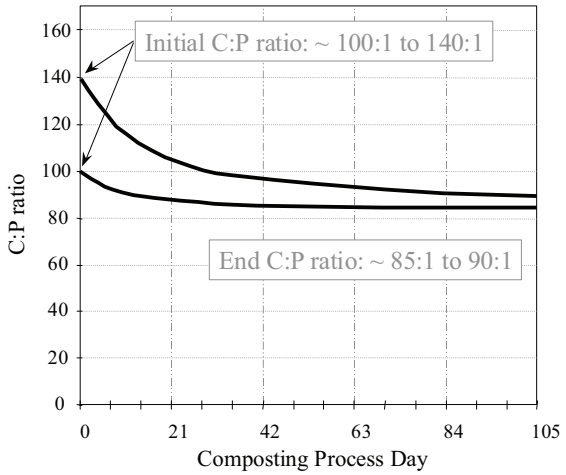

Fig 05.02-B Optimal carbon to phosphorus ratio change over time through the composting process.

Test Method: Indicator Ratios. Ammonium to Nitrate Ratio							**Units:** unitless	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.02-C		05.02-C				05.02-C

05.02-C AMMONIUM TO NITRATE RATIO

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

12. Calculations for Method C

12.1 *Calculate N:N Ratio:*

$$N{:}N = [NH_4\text{-}N] \div [NO_3\text{-}N] \qquad \textit{Equation 12.1}$$

where:

N:N = Ammonium to Nitrate ratio, unitless,

$[NH_4\text{-}N]$ = concentration of ammonium nitrogen, mg kg^{-1}, refer to *Method 04.02-C Ammonium Determination*, and

$[NO_3\text{-}N]$ = concentration of nitrate nitrogen, mg kg^{-1}, refer to *Method 04.02-B Nitrate Nitrogen Determination.*

13. Interpretation of Results for Method C

13.1 *Water-Soluble Nitrogen Concentrations*—The NH_4-N:NO_3-N ratio has little value and should not be considered a valid Group B parameter to establish a Compost Maturity Index Rating for composts with very low concentrations of both NH_4–N and NO_3–N (including NO_2–N), i.e., when their sum is less than approximately 75 to 100 mg kg^{-1} dw. Refer to Method 05.02-G CCQC Maturity Index for additional maturity indices.

13.2 As composting progresses, ammonia is oxidized (transformed) to nitrate as microbial activity converts organic carbon and other compounds into CO_2 and H_2O. During later stages of the composting process as stability increases and the compost matures, nitrate often becomes the dominant form of water-soluble nitrogen.

13.3 Information concerning the ammonium to nitrate ratio as an indicator of maturity for different feedstock blends and throughout the composting process is being sought to develop an interpretation statement.

Test Method: Indicator Ratios. Carbon to Sulfur Ratio							**Units:** unitless	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.02-D		05.02-D				05.02-D

05.02-D CARBON TO SULFUR RATIO

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

14. Calculations for Method D

14.1 *Calculate C:S Ratio:*

$$C:S = C \div S \qquad \textit{Equation 14.1}$$

where:

C:S = Carbon to Sulfur ratio, unitless,
C = percent organic carbon, %, and
refer to *Test Method 04.01 Organic Carbon*, and
S = concentration of sulfur (total), %,
refer to *Method 04.5-S Sulfur*.

15. Interpretation of Results for Method D

15.1 As composting progresses, elemental sulfur and sulfide (oxidation state –2) is oxidized (transformed) to form sulfites (oxidation state +4) and sulfates (oxidation state +6). Possible candidates for this process include the bacteria of *Thiobacilllus spp.* that use inorganic sulfur compounds as a source of energy during respiration. The anerobic microbes include *T. thiooxidans* which is a strict chemoautotroph and is active at pH 3 and lower, and *T. thioparus* which is acid sensitive and active at neutral pH. *T. novellus*, which is also active at neutral pH, cannot use elemental sulfur, but oxidizes organic compounds as well as inorganic salts during respiration. With the exception of *T. novellus* all species are obligate autotrophs and derive no energy from oxidation of organic carbon. Bicarbonate or CO_2 supplies carbon for chemoautotrophic growth. Provided the physical conditions are optimum, (e.g., pH, aeration and supply of CO_2), these organisms should be active and able to convert much of the sulfur to sulfate. During later stages of the composting process as stability increases and the compost matures, the carbon to sulfur ratio will decrease as carbon is respired as CO_2 and more sulfate forms.

15.2 Information concerning applications for the carbon to sulfur ratio is being sought to develop an interpretation statement.

Test Method: Indicator Ratios. Cadmium to Zinc Ratio						**Units:** unitless		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							05.02-E	

05.02-E CADMIUM TO ZINC RATIO

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTED BY—Dr. Rufus Chaney, USDA-BARC; Beltsville, MD.

16. Calculations for Method E

16.1 *Calculate Cd:Zn Ratio:*

$$Cd:Zn = Cd \div Zn \qquad \textit{Equation 16.1}$$

where:

Cd:Zn = Cadmium to Zinc ratio, unitless,

Cd = concentration of cadmium, mg kg^{-1} dw, and refer to *Test Method 04.06-Cd Cadmium*, and

Zn = concentration of zinc, mg kg^{-1} dw, and refer to *Test Method 04.06-Zn Zinc.*

17. Interpretation of Results for Method D

17.1 Assessment of the ultimate bioavailability to humans of soil Cd transferred through foods is very complex. As long as Cd:Zn of crops is near the natural level of less than 0.01, most livestock and wildlife have no increase of Cd in tissues used as food even when crop Cd is increased substantially, i.e., within limits imposed by Zn phytotoxicity and inhibition of Cd transport to grain by Zn. Crop Zn inhibits Cd absorption and/or retention in animal tissues, even liver and kidney (Reeves and Chaney, 2001; McKenna et al., 1994).

Table 05.02-G1 Cd:Zn Interpretation Guide.

Cd:Zn Ratio	Indication of Cd Bioavailability*
< 0.01	not bioavailable
> 0.01	potentially bioavailable

* Cd:Zn ratio interpretation is provided for use with compost to be applied on crops, excluding rice, AND with a Zn concentration < 500 mg kg^{-1}.

17.2 Soil factors found to influence plant uptake of soil Cd include: pH; Cd; Cd:Zn ratio; chloride; levels of metal sorbents such as hydrous Fe and Mn oxides and organic matter. Agronomic factors that can influence Cd uptake include: form of N applied; previous crop; Cd level in P fertilizers; etc.

17.2.1 Zn provides protection against Cd transfer to foods by its potential for phytotoxicity and inhibition of Cd uptake and translocation in plants. Because Zn and Cd are accumulated by plants in about the ratio they occur in acidic soils with elevated Zn and Cd, the maximum foliar Cd concentration is limited by Zn phytotoxicity. If the crop is seriously harmed (e.g., 500 mg Zn kg^{-1} and only 5 mg Cd kg^{-1}), the cause would be characterized and limestone added to reduce Zn uptake and toxicity. Thus Zn limits maximum crop Cd concentration, and protects the food-chain. There can be no invisible Cd poisoning with most plant species when soil Cd:Zn remains below approximately 0.01, i.e., 1:100.

17.2.2 Most plant species exclude Cd relative to Zn during formation of grain, fruits, or storage roots. However, rice grown in flooded soils has an opposite pattern in which grain Cd is substantially increased, while grain Zn remains at background levels. ZnS and CdS are formed in flooded soils, but Cd S is oxidized rapidly upon drainage promoting uptake. The exclusion of Zn from grain of rice grown in flooded soils was reported for the original case of *itai-itai* disease in Japan; rice grain was obtained from different villages as part of the epidemiological studies (Fukushima et al., 1973).

17.3 The ultimate risk from bioavailable crop Cd is a suggested basis for any limits developed for Cd in crops, soils, fertilizers and soil amendments such as biosolids and composts.

17.3.1 Evidence suggests that interactions between Cd bioavailability and an adequate nutritional status result in the exclusion of feed Cd by livestock (Bray et al., 1985; Kienholz et al, 1979). This research supports the assertion that humans are well protected from Cd in food and feed crops other than rice and tobacco.

17.3.2 It is apparent that misunderstandings about the agronomy of Cd and Zn, and the ability of rice to exclude Zn from grain, have caused over-estimation of risk from soil and food Cd; Fe, Zn and Ca in rice are frequently deficient for subsistence human diets, increasing risk.

17.3.3 The high Cd:Zn of P-fertilizers may cause this Cd to have higher food-chain mobility and bioavailability than other Cd sources reaching cropland, but tests of this specific question have yet to

be reported. Evidence does suggest that high Cd P-fertilizers should not be used to fertilize land used to produce rice or tobacco.

17.3.4 Additional feeding tests with crops grown on soils with typical Cd contamination are needed to more thoroughly understand how unusual rice is in accumulating bioavailable Cd as compared to other important foods, and to better characterize the biochemistry of Fe- and Zn-inhibition of Cd absorption by animals.

Test Method: Indicator Ratios. Agricultural Index						**Units:** ratio: % $\%^{-1}$ dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5: Compost Curing*	*Step 6: Compost Screening and Refining*	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
05.02-F	05.02-F				05.02-F	05.02-F		05.02-F

05.02-F AGRICULTURAL INDEX

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTED BY—Frank Shields, Soil Control Laboratory – Watsonville, CA

18. Calculation for Method F

18.1 Elemental (total) determinations required for the following equation include nitrogen (TN, or TKN+NO_3^-–N, or TKN with NO_3 reduction step), phosphorus (P), potassium (K), chlorine (Cl) and sodium (Na). Refer to the method for each element indicated.

18.1.1 This calculation is used to identify optimal feedstock blends. It may also be used to rate or classify a finished compost product.

18.2 *Convert Elemental P and K to P_2O_5 and K_2O:*

P_2O_5 = P × 2.2914 *Equation 18.2.1*

K_2O = K × 1.2046 *Equation 18.2.2*

where:

P = Elemental (total) phosphorus, refer to Method 04.03-A, %,

K = Elemental (total) potassium, refer to Method 04.04-A, %,

P_2O_5 = Fertilizer industry reporting standard for phosphorus content, %, and

K_2O = Fertilizer industry reporting standard for potassium content, %.

18.3 *Calculate AgIndex:*

AI = [N + P_2O_5 + K_2O] ÷ [Na + Cl_2] *Equation 18.3*

where:

AI = AgIndex, unitless ratio,

N = Elemental (total) nitrogen, refer to Method 04.02-A, %,

P_2O_5 = Fertilizer industry reporting standard for phosphorus content, %, and

K_2O = Fertilizer industry reporting standard for potassium content, %.

Na = Elemental (total) sodium, refer to Method 04.05-Na, %, and

Cl_2 = (total) chloride, refer to Method 04.05-Cl, %.

19. Interpretations for Method F

19.1 The interactions of edaphic, climatic and management factors, (e.g., soil physical, chemical and biological characteristics, soil water chemistry, crop rotation scheme, quantity and frequency of compost applications, etc.), influence the ultimate impact of applying a high sodium and/or chloride compost to the soil. Interpretation guidelines for common edaphic conditions are presented in Fig 05.02-E1.

19.2 It is suggested that composts with an *AgIndex* below two may cause salt injury to a susceptible crop. An *AgIndex* above ten indicates that high levels of Na and Cl are not limiting factors, while the primary nutrients, i.e., N, P_2O_5 and K_2O, may be the principal limiting factors. A compost with an *AgIndex* between two and ten requires more thorough interpretation of the soil conditions where the compost is to be applied. An *AgIndex* of two to five is acceptable for applications on highly permeable soils (sandy) with good water quality and low salt concentrations. An *AgIndex* value above five is acceptable for application on soils with poor drainage and/or poor water quality, or high Cl_2 (>50 mg kg^{-1}) and/or Na (>150 mg kg^{-1}) concentrations.

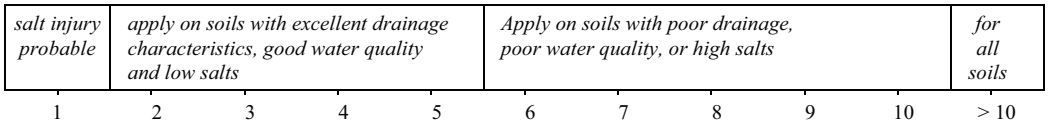

Fig 05.02-F1 AgIndex interpretation and use guidelines for common edaphic conditions.

Test Method: Indicator Ratios. CCQC Maturity Index						**Units:** unitless index		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
						05.02-G		05.02-G

05.02-G CCQC MATURITY INDEX

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTED BY—The California Compost Quality Council (CCQC) Stability/Maturity Oversight Committee[1].

20. Interpretations for Method G

20.1 *Maturity Rating*—Compost is tested and classified as "very mature, mature, or immature" according to the Compost Maturity Index.

20.2 The compost maturity index is implemented using a three-tier decision process as illustrated in Figure 05.02-G1.

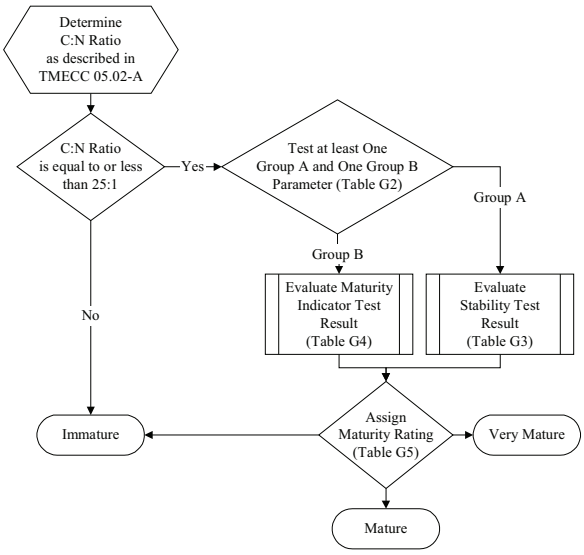

Figure 05.02-G1. Compost maturity assessment process.

Table 05.02-G1 Compost maturity index.

VERY MATURE	MATURE	IMMATURE
Well cured compost	Cured compost	Uncured or raw compost
No continued decomposition	Odor production not likely	Odor production likely
No toxicity potential	Limited toxicity potential	High toxicity potential
No impact on plant-available soil nitrogen	Minimal impact on plant-available soil nitrogen	Significant impact on plant-available soil nitrogen

Table 05.02-G2. Compost maturity index parameters.

Carbon Nitrogen Ratio (C:N, TMECC 05.02-A)	
Group A *(Stability)*	Group B *(Maturity)*
Respirometry Tests (TMECC 05.08): ▪ Specific Oxygen Uptake Rate (TMECC 05.08-A); ▪ Carbon Dioxide Evolution Rate (TMECC 05.08-B); ▪ Dewar Self-Heating Test (TMECC 05.08-D); ▪ Solvita CO_2 (TMECC 05.08-E); and/or ▪ Biologically Available Carbon (TMECC 05.08-F)	Ammonium (TMECC 04.02-C); NH_4-N:NO_3-N Ratio[2] (TMECC 05.02-C); Biological Assays (TMECC 05.05): ▪ Emergence and Seedling Vigor ▪ In-Vitro Germination and Root Elongation, or ▪ Earthworm Bioassay: The Minnesota "Z"-Test; Solvita NH_3 (TMECC 05.08-E); and/or Volatile Fatty Acids (TMECC 05.10-A)

CAUTION !—Anticipate continued refinement of the numerical thresholds presented in Tables 05.02-G3 and 05.02-G4. A Maturity Index should never be the sole indicator for determining compost end use. Compost application instructions should consider multiple compost analytical parameters, (e.g., pH, soluble salts, sieve size, nutrient content, metals content, pathogens, AgIndex, etc.).

[1] The CCQC Maturity Index was developed under a contract with the California Integrated Waste Management Board. CIWMB Project Manager - Mike Leaon; and CCQC Project Manager - Matthew Cotton, Integrated Waste Management Consulting, Nevada City, California. The Maturity Index evolved from the CCQC Laboratory Practices Committee Chaired by Dr. Marc Buchanan, Buchanan Associates, Scotts Valley, California. Committee members included: William F. Brinton, Woods End Laboratories, Mt. Vernon, Maine; Frank Shields, Soil Control Laboratory, Watsonville, California; James West, Soil and Plant Laboratory, Santa Clara California; and Wayne H. Thompson, Edaphos International, Houston, TX.

[2] For composts with very low concentrations of both NH_4–N and NO_3–N (including NO_2–N), i.e., their sum is less than approximately 75 to 100 mg/kg dw, the NH_4-N:NO_3-N ratio has little value and should not be considered a valid Group B parameter to establish a Compost Maturity Index Rating.

20.3 *Compost Stability*—At least one respirometry method is selected and the test outcome is evaluated according to the thresholds presented in Table 05.02-G3 and a stability rating assigned.

Table 05.02-G3. Stability indicator thresholds using respirometry.

Group A *(Stability)*	*Rating*		
	Very Stable	*Stable*	*Unstable*
Specific Oxygen Uptake Rate (mg O_2 per g OM per d)	< 3	3 – 10	> 10
Carbon Dioxide Evolution Rate (mg CO_2-C per g OM per d)	< 2	2 – 4	> 4
Dewar Self-Heating Test (Dewar Index)	V		< V
Headspace Carbon Dioxide (color-code for Solvita CO_2)	7 – 8	5 – 6	1 – 4
Biologically Available Carbon (mg CO_2-C per g OC per d)	< 2	2 – 4	> 4

ADAPTED FROM—TMECC Table 05.08-1 Compost stability index.

20.4 *Maturity Indicators*—At least one maturity indicator is selected and the test outcome is evaluated according to the thresholds presented Table 05.02-G4.

Table 05.02-G4. Maturity indicator thresholds.

Group B *(Maturity Indicator)*	*Rating*		
	Very Mature	*Mature*	*Immature*
Ammonium, (mg kg^{-1} dw)	< 75	75 - 500	> 500
Ammonium:Nitrate Ratio[3], (unitless ratio)	< 0.5	0.5 – 3.0	> 3.0
Seedling Emergence, (% of control), AND Seedling Vigor, (% of control)	> 90 and > 95	80 – 90 and 85 – 95	< 80 and < 85
In-Vitro Germination and Root Elongation , (% of control)	> 90	80 – 90	< 80
Earthworm Bioassay: The Minnesota "Z"-Test (% weight gain)	< 20	20 – 40	> 40
Ammonia, (color-code for Solvita NH_3)	5	4	3 – 1
Volatile Fatty Acids, (mmoles g^{-1} dw)	< 200	200 – 1,000	> 1,000

20.5 *Maturity Assessment*—A compost is assigned a maturity rating of immature, mature, or very mature, pending the outcome of up to three parameters analyses. The compost C:N ratio is first evaluated: a compost with a C:N ratio greater than 25:1 would be classified as immature compost; no further testing would be necessary needed for the maturity classification. If the C:N ratio is equal to or less than 25:1, then the compost must be evaluated for both stability using one of the parameters listed in Group A (Table 05.02-G3), and for maturity using one of the indicators presented in Group B (Table 05.02-G4). All possible maturity assessment outcomes are presented in Figure 05.02-G2.

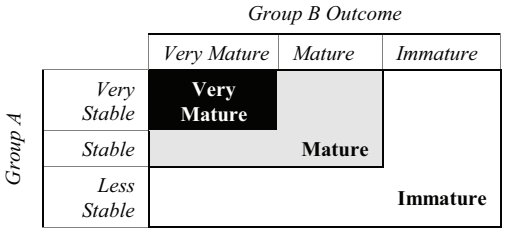

Figure 05.02-G2. Maturity assessment matrix. Applied when the C:N ratio is equal to or less than 25:1.

[3] NO_3-N represents a sum of both nitrite and nitrate forms of nitrogen.

05.02 METHODS SUMMARY

21. Report

21.1 *Ratios (Methods 05.02-A through 05.02-E):*

21.1.1 *Digest and Determinations*—Report sample digest method and determination technique for each element; report the material or matrix type, (e.g., compost, feedstock, etc.); report the material source, (e.g., mixed municipal solid waste, source separated municipal solid waste, biosolids, yard waste, manure, etc.); and report the type and relative amount of chemical amendments added to the compost product.

21.1.2 *Units*—unitless ratio, report to ±0.1.

21.1.3 *Significant Figures*—Report ratio to three significant figures.

21.2 *Indices (Methods 05.02-F and 05.02-G):*

21.2.1 Report results for each paramerter considered, and corresponding test methods, to establish the index rating.

21.2.2 Report the index with interpretative information that is provided in figures and tables.

21.2.3 *Digest and Determinations*—Report sample digest method and determination technique for each element; report the material or matrix type, (e.g., compost, feedstock, etc.); report the material source, (e.g., mixed municipal solid waste, source separated municipal solid waste, biosolids, yard waste, manure, etc.); and report the type and relative amount of chemical amendments added to the compost product.

22. Precision and Bias

22.1 *Method 05.02-A Carbon to Nitrogen Ratio*—The precision of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

22.1.1 Organic carbon was determined by Method 04.01-A and total nitrogen was determined by Method 04.02-D, 19.3.1.2. Total Kjeldahl Nitrogen plus NO_3-N for TN.

22.1.2 Precision was determined for 10 subsamples taken from a field composite sample for each of three sites at two separate sampling periods (1993). Variability is expressed as coefficient of variation (%CV, standard deviation ÷ mean × 100).

Table 05.02-A1 Carbon to nitrogen ratio precision.

% CV	*Number of Samples*
10.9	10
4.9	10
8.7	10
4.5	10
6.1	10
10.7	10

22.2 *Method 05.02-B Carbon to Phosphorus Ratio*—The precision of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

22.2.1 Organic carbon was determined by Method 05.07-A and total phosphorus was determined by Method 04.06-A (ICP-AES) and digested using Method 04.08-A (US EPA method 3051 modified for compost).

22.2.2 Precision was determined for ten subsamples taken from a field composite sample for each of three sites at two separate sampling periods (1993). Variability is expressed as coefficient of variation (%CV, standard deviation ÷ mean × 100).

Table 05.02-B1 Carbon to phosphorus ratio (C:P) precision.

% CV	*Number of Samples*
10.5	10
8.1	10
14.5	10
6.7	10
7.3	10
14.4	10

22.3 *Method 05.02-C Ammonium to Nitrate Ratio*—The precision of this test was determined by the Research Analytical Laboratory, Department of Soil, Water, and Climate; University of Minnesota for the MN-OEA CUP Project, 1993-1994. St. Paul, MN. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

22.3.1 Nitrate-N and ammonium-N were determined by colorimetry with Methods 04.02-B and C, respectively.

22.3.2 Precision was determined for 10 subsamples taken from a field composite sample for each of three sites at two separate sampling periods (1993). Variability is expressed as coefficient of variation (%CV, standard deviation ÷ mean × 100).

Table 05.02-C1 Ammonium to Nitrate Ratio (N:N) precision.

% CV	*Number of Samples*
24.5	10
3.1	10
34.8	10
12.6	10
24.9	10
2.8	10

22.4 *Method 05.02-D Carbon to Sulfur Ratio*—The precision and bias of this test has not been determined. Data are being sought for use in developing a precision and bias statement.

22.5 *Method 05.02-E Cadmium to Zinc Ratio*—The precision and bias of this test has not been determined. Data are being sought for use in developing a precision and bias statement.

22.6 *Method 05.02-F Agricultural Index*—The precision and bias of this test has not been determined. Data are being sought for use in developing a precision and bias statement.

22.7 *Method 05.02-G CCQC Maturity Index*—The precision and bias of this test has not been determined. Data are being sought for use in developing a precision and bias statement.

23. Keywords

23.1 ammonia; ammonium; cadmium; denitrification; iron; maturity index; nitrate; nitrification; nitrogen; total nitrogen; kjeldahl nitrogen; organic nitrogen; nitrate reduction; stability index; sulfur; phosphorus; organic carbon; carbon; Agricultural Index; AgIndex; chloride; limiting factors; nutrients; phosphorus; potassium; salt; salt index; sodium; zinc

Test Method: Color. One Method.						**Units:** unitless index		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.03-A	05.03-A	05.03-A		05.03-A		05.03-A

05.03 COLOR

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This test is appropriate for use as a teaching-aid, i.e., K-12. It covers a method to make qualitative estimates of compost stability and maturity using color as the principal indicator.

1.1.1 *Method 05.03-A Field Approximation of Compost Color and Odor.*

NOTE 1—This field test was devised by E&A Environmental Consultants, Inc. to be performed in conjunction with Method 05.06-A.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 05.06-A Compost Odor Assessment.

2.2 *Other References:*

Munsell Color Company, Inc. Munsell soil color charts: hues - 7.5R thru 5Y. Special form for use of soil scientists, geologists, archaeologists. Munsell Color Co., Inc., Baltimore, Md. [1950]

3. Terminology

3.1 *color, n*—That aspect of things caused by differing qualities of the light reflected or emitted by them, definable in terms of the observer or of the light. Appearance of objects or light sources described in terms of the individual's perception of them, i.e. hue, lightness, and saturation for objects and hue, brightness, and saturation for light sources. The characteristics of light, by which the individual is made aware of objects or light sources through the receptors of the eye, are described in terms of dominant wavelength, luminance, and purity. Refer to Table 05.03-A1 Rating system for categorizing compost color and Fig 05.03-A1 Compost color assessment chart.

3.2 *humus, n*—A brown or black organic substance consisting of partially or wholly decayed vegetable or animal matter that provides nutrients for plants and increases the ability of compost to retain water.

3.3 *maturity, n*—An organo-chemical state of the compost which indicates the presence or absence of organic phytotoxins in stable to very stable compost. Organic phytotoxins can include any or all volatile fatty acids, certain heavy metals, and ammonia or ammonium nitrogen. Phytotoxic conditions can include extreme pH, salts concentration, and anaerobosis.

3.4 *odor, n*—The property or quality of a thing that affects, stimulates, or is perceived by the sense of smell. Refer to Table 05.03-A1, Odor assessment and rating chart.

3.5 *smell, v*—To perceive the scent of (something) by means of the olfactory nerves.

3.6 *stability, n*—Stage in the composting process where microbial activity diminishes with the corresponding decrease of available organic carbon and other energy sources. Stability of the compost may be manifest in relative changes in compost color. At the early stages of composting, the rate of change in biological activity is relatively large and the overall color of the material tends to be light brown. As the biological activity declines, the compost becomes darker, and finally becomes dark brown or black as the rate of change in biological activity changes imperceptibly.

3.7 *stability index, n*—the level of microbial activity in a sample based upon measurement of respiration (refer

to Method 05.08 Respirometry, Table 05.08-1 Compost Stability Index).

4. Summary of Test Methods

4.1 *Method 05.03-A Field Assessment of Compost Color*—This method uses a color template and odor descriptions for determining color and odor values of a compost sample. The resulting values are matched to a color/odor matrix that defines stable and unstable compost. This test was designed to be performed in conjunction with Method 05.06-A Field Assessment of Compost Odor.

4.1.1 Determining the color of compost is used for field diagnostics to aid in approximating compost stability. The color of a representative sample is compared to those in the compost color assessment chart; there are five shades of brown, from light to dark.

4.1.2 A sample is color classified by assigning a value from one to five. The chart color is matched to that most closely resembling compost color. The chart color value (1, dark through 5, light) is assigned to the compost sample.

5. Significance and Use

5.1 Compost color deepens as compost matures. The process of color change is initiatd when soluble organic componds are oxidized during the thermophilic phase of the composting process. High temperature promotes color change. Thermophilic temperatures [up to 60°C] generally occur during the active composting phase. Colors will deepen further during the curing phase of the composting process as more complex polymeric organic constituents are oxidized and humic compounds accumulate.

5.2 Color change is relative, where the color of the compost reflects its composition. Color is sometimes used as a quick and easy way to indicate the stability status of a compost product. It is useful when the producer is experienced and/or the feedstock and composting technology are known.

6. Interference and Limitations

6.1 *Method 05.03-A Field Assessment of Compost Color*—This test should not be used for inspecting and rejecting loads of compost delivered to a site. Color should only be used to indicate compost maturity if the producer is experienced and the feedstock and composting technology are known.

6.2 Color varies enormously between composts of similar origin and within compost groups based on fluctuations in ingredients, water content, degree of comminution, (size reduction), and state of aeration. CAUTION! **Color comparisons should be made only within the same compost batch over time.**

6.2.1 There is no published evidence that color *per se* relates to stability or maturity. The method should be used with great caution.

NOTE 2—In technologies that grind inert material into fine particles, especially glass, samples of finished compost product exhibit a significantly lighter brown color even though the organic matter in the compost is stable or very stable.

7. Sample Handling

7.1 *Method 05.03-A Field Assessment of Compost Color and Odor*—This test calls for as-received moist, unsieved material, sealed in a temporary storage container, (e.g., plastic Ziploc®-type container).

Test Method: Color. *Quick-Test* for Field Assessment of Compost Color and Odor							**Units:** unitless	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
		05.03-A	05.03-A	05.03-A		05.03-A		05.03-A

05.03-A FIELD ASSESSMENT OF COMPOST COLOR AND ODOR

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Apparatus for Method A

8.1 *None required.*

9. Reagents and Materials for Method A

9.1 *Paper*—white, paper dimension is optional.

9.2 Sample Containers—disposable plastic bag or container, (e.g., Ziploc® storage containers, etc.).

10. Procedure for Method A

10.1 *Odor Assessment:*

10.1.1 Open the sample container containing the sample.

10.1.2 Place your nose near the opening.

10.1.3 Smell the compost sample.

10.1.4 Assess and rate odor following the odor descriptions listed in Table 05.03-A1 Odor assessment and rating table.

Table 05.03-A1 Odor assessment and rating table.

Odor Rating	*Odor Characteristic*
1	Earthy, soil-like, no odor
2	Moldy, musty, mildew, swampy
3	Fruity, sweet, black licorice, slight pine, slight ammonia, tobacco, burnt odor
4	Sour, rotting grass, manure, sour milk, vinegar, strong ammonia, turpentine, urine
5	Fresh yard debris, wet leaves, hay, strong pine odor

10.2 *Color Assessment:*

10.2.1 Transfer approximately 50 cm^3 of the compost sample onto a white paper sheet.

10.2.2 Assess compost color with a Munsell color chart using values listed in Table 05.03-A2. **(NEED REFERENCE OR SOURCE FOR MUNSELL COLOR CHART)**

10.2.2.1 Match the compost color to one color from the chart and assign the corresponding chart value to the compost sample.

10.2.3 Record the chart color value of the sample. If more than one chart color matches the compost color, assign the highest chart value to the sample.

Table 05.03-A2 Rating system for categorizing compost color.

Color Rating	Color Characteristic	Munsell Chart Value	Color
1	Black, very dark brown	gley gley 5YR 2.5/1	3/1, 2.5/1,
2	Dark brown	5YR 5YR 5YR 3/2	3/4, 3/3,
3	Medium brown	5YR 5YR 5YR 4/3	4/6, 4/4,
4	Light brown	7.5YR 7.5YR 7.5YR 4/3	4/6, 4/4,
5	Yellow-green	2.5Y 2.5Y 2.5Y 6/2	6/4, 6/3,

10.3 Interpret results from steps 10.1 and 10.2 and assign field approximation of maturity using Fig 05.03-A1.

CAUTION !—Employ caution when attempting field assesment of maturity using this test. Compost color change is merely a relative indicator and varies significantly with feedstock types and blends, and light source and quality.

11. Interpretation of Results for Method A

11.1 Assess the maturity of the compost by matching the resulting odor (step 10.2) and color (step 10.3) values in the compost maturity approximation matrix presented in Fig 05.03-A1.

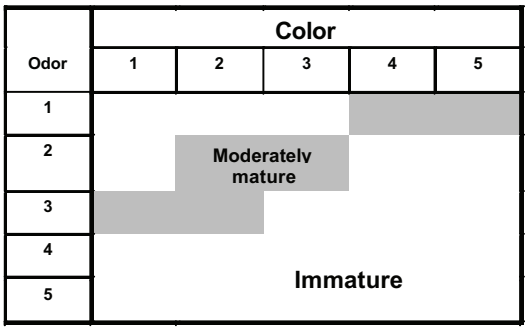

Fig 05.03-A1 Compost maturity approximation matrix.

11.2 *Field Approximation for Maturity:*

11.2.1 *Very Mature*—The assessed sample values merge in the black zone of the maturity matrix.

11.2.1.1 The compost represented by that sample may be stable and mature.

11.2.2 *Moderately Mature*—The assessed sample values merge in the gray transition zone of the maturity matrix.

11.2.2.1 Repeat steps 10.2 and 10.3 with a different compost sample.

11.2.2.2 Repeat steps 10.2 and 10.3 with a different person.

11.2.3 *Immature*—Assessed sample values merge in the white zone of the maturity matrix.

11.2.3.1 Repeat steps 10.2 and 10.3 with a different compost sample.

11.2.3.2 Repeat steps 10.2 and 10.3 with a different person.

05.03 METHODS SUMMARY

12. Report

12.1 *Method 05.03-A Field Assessment of Compost Color and Odor:*

12.1.1 *Verify Field Assessments*—It is highly recommended that a sample is submitted for the following laboratory analysis: C:N ratio, NH4:NO3 ratio, respirometry test results and stability rating, germination and growth screening tests.

12.1.2 *Color*—Report sample color rating numerical value, color characteristic and Munsell color chart value for that sample. Refer to Table 05.03-A2 Rating system for categorizing compost color.

12.1.3 *Odor*—Report sample odor rating numerical value and odor characteristic description for that sample. Refer to Table 05.03-A1 Odor assessment and rating table.

12.1.4 *Field Approximation for Maturity*—Report index rating derived from Table 05.03-A2: very mature, mature, moderately immature, immature.

12.1.5 Report the material analyzed (e.g., compost, feedstocks), source materials, (e.g., municipal solid waste, biosolids, manure, yard waste, etc.), and age of compost in months.

13. Precision and Bias

13.1 *Method 05.03-A Field Assessment of Compost Color*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

14. Keywords

14.1 color; humus; maturity; stability; odor; maturity assessment matrix

Test Method: Enzyme Activity and Analysis. Five Methods						**Units:** see methods		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.04-A	05.04-A	05.04-A		05.04-A		05.04-A
		05.04-B	05.04-B	05.04-B		05.04-B		05.04-B
		05.04-C	05.04-C	05.04-C		05.04-C		05.04-C
		05.04-D	05.04-D	05.04-D		05.04-D		05.04-D
		05.04-E	05.04-E	05.04-E		05.04-E		05.04-E

05.04 ENZYME ACTIVITY AND ANALYSIS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers the determination of enzyme activity and enzyme content in compost.

1.1.1 *Method 05.04-A Phosphatases.*

1.1.2 *Method 05.04-B Dehydrogenases*.

1.1.3 *Method 05.04-C Proteases.*

1.1.4 *Method 05.04-D Cellulases.*

1.1.5 *Method 05.04-E Peroxidases.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *Enzyme Handbook. vol 1-12—Springer-Verlag Berlin Heidelberg:*

2.1.1 The Enzyme Handbook is a concise and complete description of more than 3,000 enzymes. The description of each enzyme is divided into the following sections: nomenclature, reaction and specificity, enzyme structure, isolation/preparation, stability, cross references and literature references. The volumes are:

Enzyme Handbook 1, Class 4: Lyases Eds. D. Schomburg, M. Salzmann 1990. Approx. 810 pp. Loose-Leaf-Binder ISBN 3-540-52579-3;

Enzyme Handbook 2, Class 5: Isomerases, Class 6: Ligases Eds. D. Schomburg, M. Salzmann 1990. Approx. 810 pp. Loose-Leaf-Binder ISBN 3-540-52580-7;

Enzyme Handbook 3, Class 3: Hydrolases Eds.: D. Schomburg, M. Salzmann 1991. Approx. 825 pp. Loose-Leaf-Binder ISBN 3-540-53729-5;

Enzyme Handbook 4, Class 3: Hydrolases Eds.: D. Schomburg, M. Salzmann 1991. Approx. 825 pp. Loose-Leaf-Binder ISBN 3-540-53730-9;

Enzyme Handbook 5, Class 3: Hydrolases Eds.: D. Schomburg, M. Salzmann 1991. Approx. 1105 pp. Loose-Leaf-Binder ISBN 3-540-54209-4;

Enzyme Handbook 6, Class 1: Oxidoreductases Eds.: D. Schomburg and M. Salzmann 1993. Approx. 950 pp. Loose-Leaf-Binder ISBN 3-540-56435-7;

Enzyme Handbook 7, Class 1.5-1.12: Oxidoreductases. Eds.: D. Schomburg, M. Salzmann, D. Stephan 1993. Approx. 800 pp. Loose-Leaf-Binder ISBN 3-540-57246-5;

Enzyme Handbook 8, Class 1.13 - 1.99: Oxidoreductases Eds.: D. Schomburg, D. Stephan 1994. Approx. 800 pp. Loose-Leaf-Binder. ISBN 3-540-57837-4;

Enzyme Handbook 9, Class 1.1.1.1 - 1.1.1.149 Oxidoreductases Eds: D. Schomburg, D. Stephan 1995. Approx. 800 pp. Loose-Leaf-Binder; ISBN 3-540-59077-3;

Enzyme Handbook 10, Class 1.1.1.150 - 1.1.99.26 Oxidoreductases Eds.: D. Schomburg, D. Stephan 1995. Approx. 800 pp. Loose-Leaf-Binder; ISBN 3-540-5949-9;

Enzyme Handbook 11, Class 2.1 - 2.3 Transferases Eds.: D. Schomburg, D. Stephan 1996. Approx. 700 pp. Loose-Leaf-Binder; ISBN 3-540-60295-X Enzyme

Handbook 12 Eds.: D. Schomburg, D. Stephan 1996 (in press).

Enzyme Nomenclature. 1992. Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes. Prepared by Edwin C. Webb for NC-IUBMB. Academic Press, San Diego.

2.2 *Other References:*

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Tabatabai, M.A. 1982. Soil Enzymes. In: Methods of Soil Analysis, Part II. Chemical and Microbial Properties. pp 903-947, ASA-SSSA, Madion, WI.

3. Terminology

3.1 *N-acetyl-β-glucosaminidase, n*—(EC 3.2.1.30). This enzyme catalyzes the hydrolysis of terminal, non-reducing N-acetyl-beta-glucosamine residues in chitobiose and higher analogs and in glycoproteins. Active in pH range 4-6.

3.2 *arylsulfatase, n*—(EC 3.1.6.1). This enzyme catalyzes the hydrolysis of a phenolsulfate producing phenol and sulfate. Important in sulfur metabolism within cells.

3.3 *L-asparaginase, n*—(EC 3.5.1.1). Also known as L-asparagine amidohydrolase. This enzyme catalyzes the hydrolysis of L-asparagine, producing L-aspartate and ammonia. Active within pH range of 4-10 and at temperatures up to 60°C.

3.4 *amidase, n*—(EC 3.5.1.4). Also known acylamide amidohydrolase. This enzyme catalyzes the hydrolysis of monocarboxylic acid amide producing monocarboxylate and ammonia. Active at temperatures up to 50°C.

3.5 *cellulase, n*—Any of several enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze the hydrolysis of cellulose and other plant polysaccharides. The hydrolysis of native cellulose is achieved by the cooperative activity of two enzymes namely: cellulase (EC 3.2.1.4) and exo-1-4β -D glucanase. Cellulase catalyses the endohydrolysis of 1.4-beta-D-glucosidic linkages.

3.6 *dehydrogenase, n*—A group of mostly endocellular enzymes which participate in the metabolic reactions (e.g., citric acid) producing energy in the form of ATP through the oxidation and fermentation of glucose.

3.7 *enzyme, n*—Any of numerous proteins or conjugated proteins produced by living organisms and functioning as biochemical catalysts.

3.8 *Enzyme Commission Number (EC), n*—Nomenclature system proposed by the International Union of Biochemistry and Molecular Biology. The first number shows to which of the six main divisions the enzyme belongs, where 1, 2, 3, 4, 5 and 6 represent the classes: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases, respectively. The second number indicates the subclass, the third number

the sub-subclass and the fourth number is the serial number of the enzyme in its subclass. Refer to Enzyme Nomenclature. 1992. Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes. Prepared by Edwin C. Webb. Academic Press San Diego.

3.9 *β-glucosidase, n*—(EC.3.2.1.21). Also known as Beta-D-glucoside glucohydrolase. This enzyme catalyzes the hydrolysis of beta-D-oligosaccarides producing glucose. Examples of a natural substrate is cellobiose in cellulose degrading organisms.

3.10 *β-galactosidase, n*—Also known as lactase. This enzyme catalyzes the hydrolysis of terminal non-reducing *B-D*-galactose residues in *B-D*-galactosides. A natural substrate for this enzyme is lactose. It is adsorbed and hydrolyzed in the intestine.

3.11 *laccase, n*—(EC 1.10.3.2). Also known as oxidoreductase. Laccase catalyzes the oxidation of benzenediol producing benzosemiquinone. Oxidizes several natural substrates including *p*-cresol, catechol and 1-napthol. It is involved in the decomposition of lignin by white and brown rot fungi.

3.12 *lignin peroxidase* I*, n*—Also known as ligninase, diarylpropane peroxidase or hydrogen-peroxide oxidoreductase. This is a hemoprotein that catalyzes the oxidative cleavage of C-C bonds in a number of compounds and oxidizes benzyl alcohols to aldehydes and ketones. It is involved in the oxidative breakdown of lignin in white rot basidiomycetes.

3.13 *manganese peroxidase, n*—(EC 1.11.1.13). This enzyme catalyzes the redox reaction: $Mn(II) + 2H^+ + H_2O_2 \rightarrow Mn(III) + 2H_2O$. It is involved in the decomposition of lignin by white and brown rot fungi. Manganese peroxidase acts together with lignin peroxidase in lignin degradation of white-rot fungi, the product Mn(III) is involved in the oxidative degradation of lignin in white rot basidiomycetes.

3.14 *phosphatase, n*—Any of numerous enzymes that catalyze the hydrolysis of esters and anhydrides of phosphoric acid. They are important in the absorption and metabolism of carbohydrates, nucleotides, and phospholipids and in the calcification of bone. These enzymes are classified into five major groups. These include the phosphoric monoester hydrolases (EC 3.1.3.) the phosphoric diester hydrolases (EC 3.1.4), triphosphoric monoester hydrolases (EC 3.1.5), enzymes acting on phosphoryl-containing anhydrides (EC 3.6.1) and enzymes acting on P-N bonds e.g. phosphoamidases (EC 3.9.1.1)

3.15 *phosphoric monoester hydrolase, n*—(EC 3.1.3) This is also known as acid and alkaline phosphatase. The acid phosphatase is present in acidic composts the alkaline phosphatase is present in alkaline composts. It catalyzes the overall reaction of the type: $OPOHOR_1OH + H_2O$ $OPOHOHOH + R_1OH$, where R_1 represent either alcohol or phenol groups or nucleosides.

3.16 *phosphodiesterase, n*—This enzyme is classified among the phosphoric diester hydrolases (EC 3.1.4) with an official name of orthophosphoric diester phosphohydrolase (EC 3.1.4). It catalyzes the overall reaction of the type: $OPOHOR_1OR_2 + H_2O$ $OPOHOHOR_2 + R_1OH$, where R_1 and R_2 represent either alcohol or phenol groups or nucleosides.

3.17 *protease, n*—Any of various enzymes, including the proteinases and peptidases, that catalyze the hydrolytic breakdown of proteins.

3.18 *urease, n*—(EC 3.5.1.5). Also known as urea amidohydrolase. An extrcellular enzyme that promotes the hydrolysis of urea to form carbon dioxide and ammonia. Active in pH range of 7-10 and at temperatures up to 60°C.

4. Summary of Test Methods

4.1 *Method 05.04-A Phosphatases:*

4.1.1 *Method 05.04-A1 Phosphomonoesterase (acid phosphatase)*—Phosphomonoesterase (acid phosphatase) in compost is measured by determining amount of *p*-nitrophenol released when compost is incubated with modified universal buffer (pH 6.5) at 37°C for 1 h. The *p*-nitrophenol released is extracted with an alkaline $NaOH/CaCl_2$ solution. The *p*-nitrophenol in the compost filtrate is measured colorimetrically at 400 nm and the concentration is calculated by use of a standard calibration curve.

4.1.2 *Method 05.04-A2 Phosphomonoesterase (alkaline phosphatase)*—Phosphomonoesterase (alkaline phosphatase) in compost is measured by determining amount of *p*-nitrophenol released when compost is incubated with buffered tris hydroxymethyl aminomethane (THAM) *p*-nitrophenyl phosphate solution (pH 8.0) at 37°C for 1 h. The *p*-nitrophenol released is extracted with an alkaline $THAM/CaCl_2$ solution. The *p*-nitrophenol in the compost filtrate is measured colorimetrically at 400 nm and the concentration is calculated by use of a standard calibration curve.

4.1.3 *Method 05.04-A3 Phosphodiesterase*—Phosphodiesterase activity in compost is measured by determining the amount of *p*-nitrophenol released when compost is incubated with buffered tris hydroxymethyl

aminomethane (THAM) bis-*p*-nitrophenyl phosphate solution (pH 8.0) at 37°C for 1 h. The *p*-nitrophenol released is extracted with an alkaline THAM/$CaCl_2$ solution. The *p*-nitrophenol in the compost filtrate is measured colorimetrically at 400 nm and the concentration is calculated by use of a standard calibration curve.

4.2 *Method 05.04-B Dehydrogenases*—Dehydrogenase activity in compost is measured by determining the amount of triphenyl released when compost is incubated with 3% triphenyl-tetrazolium chloride at 37°C for 24 h in the dark. The triphenyl released is extracted with methanol. The triphenyl in the compost filtrate is measured colorimetrically at 485 nm and the concentration of triphenyl is calculated by use of a standard calibration curve.

4.3 *Method 05.04-C Proteases*—Protease activity in compost is measured by determining the leucine released when compost is incubated with 2 mM benzyloxycarbonyl-phenylalanyl-leucine at pH 8.0 in Tris buffer. The leucine released is reacted with ninhydrin reagent for 15 min at 100°C and the absorption of the reactant is measured colorimetrically at 570 nm. The concentration of leucine is calculated by use of a standard calibration curve.

4.4 *Method 05.04-D Cellulases*—Cellulase activity in compost is measured by determining the amount of glucose released when compost is incubated with 1% carboxymethyl cellulase in acetate buffer at 30°C for 1 h. The compost-solvent mixture is centrifuged at 17000 *g* and the supernatant decanted and reacted with anthrone agent. The absorbance of the glucose-anthrone supernatant is measured at 625 nm and the concentration of glucose is calculated by use of a standard calibration curve.

4.5 *Method 05.04-E Peroxidases*—Peroxidase activity in compost is measured by determining the amount of H_2O_2 decomposed when sterile compost supernatant is mixed with 0.06% H_2O_2 and 0.5% dianisidine in methanol. The oxidized o-dianisidine is measured at 460 nm in a spectrometer and the concentration of o-dianisidine is calculated with the molar absorbency of o-dianisidine ($1.13\ 10^4\ M^{-1}\ cm^{-1}$). The peroxidase enzymes are extracted from the compost by mixing it with 0.2 M phosphate buffer at pH 6.0 for 5 min. The mixture is centrifuged at 8000 *g* for 10 min. The supernatant is sterilized by filtering it through 0.22 μm filter.

5. Significance and Use

5.1 *Method 05.04-A Phosphatases*—Phosphatases are a group of enzymes that catalyze hydrolysis reactions of phosphate organic compounds. The phosphatase activity should be high in composts that originate from sewage sludge or biosolids that incorporate numerous phosphate detergents. Low phosphatase activity indicates that there is either heavy metal toxicity or high concentration of inorganic phosphate, both inhibit the activity of the enzymes. The activity of the enzymes is usually highest at the beginning of the composting process and decreases as inorganic phosphate is made available by microbial activity.

5.1.1 *Method 05.04-A1*—Analysis of Phosphomonoesterase-acid phosphatases are predominant in acidic composts. This enzyme's activity should be measured in acidic composts. The phosphomonoesterase activity is correlated with medium molecular weight (10^3 to 10^4) fraction of organic carbon throughout the composting process. The activity of phosphomonoesterase decreases during composting although cyclic activity (increases/decreases in activity) is expected as pools of inorganic phosphates are immobilized/ mobilized.

5.1.2 *Method 05.04-A2*—Analysis of Phosphomonoesterase-alkaline phosphatase activity is correlated with the biomass P in the compost. When added to soil it reaches a maximum in the first three months of incubation and remains constant for up to a year. Its activity is strongly dependent upon the inorganic phosphate mobilization/immobilization cycles in the soil/compost mixture. The pH optima of this enzyme is about 10 and is not a critical indicator in acidic composts.

5.1.3 *Method 05.04-A3*—Analysis of Phosphodiesterase activity is correlated with the biomass P in the compost. When added to soil it reaches a maximum in the first three months of incubation and remains constant for up to approximately one year. Its activity is strongly dependent upon the inorganic phosphate mobilization/immobilization cycles in the soil/compost mixture. The pH optima of this enzyme is about 10 and is not a critical indicator in acidic composts. The activity of the phosphodiesterase is generally 2× lower than the alkaline phosphatase. Phosphodiesterase activity need only be measured when accurate estimates of phosphatase activity in alkaline composts are required.

5.2 *Method 05.04-B Dehydrogenases*—Dehydrogenases are a group of enzymes that participate in metabolic pathways, (e.g., citric acid cycle), catalyzing the oxidation or fermentation of glucose and producing energy in the form of ATP. Dehyrogenase activity is a reliable indicator of

microbiological activity in compost. High levels of dehyrogenase activity suggest that there are considerable amounts of readily degradable material.

Table 05.04-1 Dehydrogenase activity as an indicator of anaerbobic respiration in compost:

Relative Maturity of Compost	*Activity [mg g^{-1} dw 24 h^{-1} at 37°C]*
Finished compost activity	< 100
Active composts	500-4000
Semi-aerobic composts	4000-10000
Raw biosolids, etc.	20000-50000

5.3 *Method 05.04-C Proteases*—Proteases are a group of enzymes that participate in the turnover of nitrogen in compost. They act by breaking down the peptide bonds within proteins and peptides. Addition of compost to soil increases the protease activity and this activity tends to remain higher after long incubation times (> 6 months) than in unammended soils. This increased activity is due to increased concentrations of proteins and peptides following microbial death during the composting process. Protease activity would be highest in composts derived from high protein sources such as fish, poultry, or kitchen waste, etc. Protease activity is correlated with medium molecular weight (10^3 to 10^4) fraction of organic carbon throughout the composting process.

Dehydrogenase activity is often found to correlate with carbon mineralization.

5.4 *Method 05.04-D Cellulases*—Cellulases are a group of enzymes that participate in the break down of polysaccharides. They are particularly active in the decomposition of plant cell walls and significantly contribute to the decomposition of yard waste composts. Fungi are the dominant producers of cellulases, but only the cellulases of the white-rot fungi are able to degrade all polymers of the plant cell wall (i.e., cellulose, hemicellulose and lignin).

5.5 *Method 05.04-E Peroxidases*—Peroxidases are a group of enzymes that oxidize aromatic amines, phenols and various other compounds in the presence of H_2O_2. During the reaction an electron is removed from subunits of phenolic polymers including lignin producing phenoxy radicals. These radicals then participate in oxidative coupling reactions and result in polymerization or ring hydroxylation, followed by ring fission. Peroxidase activity increases as composts mature, when the less degradable portions (e.g., lignolytic content) of compost increase. Wood rotting fungi participate in degradation of lignin and the two families of peroxidases that participate are lignin peroxidase and manganese peroxidase.

6. Sample Handling

6.1 Aliquots of compost at as-received moisture are hand sorted to remove particles of wood, glass, metal and hard plastics.

Test Method: Enzyme Activity and Analysis. Phosphatases						**Units:** μg g^{-1} dw h^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.04-A	05.04-A	05.04-A		05.04-A		05.04-A

05.04-A PHOSPHATASES

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7. Apparatus for Method A

7.1 *Spectrophotometer*—fitted with a blue filter, with facility to measure the absorbance at 400 nm.

7.2 *Volumetric Flasks*—100-mL, 250-mL, 500-mL and 1-L.

7.3 *Erlenmeyer Flasks*—50-mL and 250-mL with stoppers.

7.4 *Colorimeter Tubes*—two per experiment.

8. Reagents and Materials for Method A

8.1 *Water*—deionized, double distilled, 17 MO·cm minimum standard.

8.2 *Toluene*—reagent grade.

8.3 *Calcium Chloride Solution (0.5 M)*—Add 800 mL of double distilled water to a 1-L volumetric flask, dissolve 73.5 g of $CaCl_2 \cdot 2H_2O$ (mol wt 147.02) and dilute the solution with more water to make a final volume of 1L.

8.4 *Standard p-Nitrophenol (PN) Solution*—In a 1-L volumetric flask, dissolve 1.0 g of *p*-nitrophenol ($C_6H_5NO_3$, mol wt 139.11) in 800 mL of double distilled water. Add more water to make a final volume of 1 L. Store the solution in refrigerator.

8.4.1 *Standard p-Nitrophenol solutions containing 50, 40, 30, 20, and 10 μg of PN mL^{-1}*—Dispense 5 mL, 4, mL, 3 mL, 2 mL, and 1 mL of the standard *p*-nitrophenol solution (1 mg mL^{-1}) into 100-mL volumetric flasks, respectively. To each flask add sufficient THAM diluent to make a final volume of 100 mL.

8.5 *Method 05.04-A3 Phosphomonoesterase (acid phosphatase):*

8.5.1 *NaOH (0.5 M)*—Place 250 mL of water into a 500-mL volumetric flask and dissolve 20 g of NaOH to the flask. Add more water to a final volume of 500 mL.

8.5.2 *HCl (0.1 M)*—Place 250 mL of water into a 500-mL volumetric flask and add 4.15 mL of concentrated HCl to the flask. Add more water to make a final volume of 500 mL.

8.5.3 *Modified Universal Buffer (MUB, 0.05 M, pH 6.5)*—Add 50 mL of water to a 250-mL volumetric flask. Add 122 mL of 1 *N* NaOH to the flask. Dissolve 3.025g of tris(hydroxymethyl) aminomethane; 2.90 g of maleic acid, 3.50 g of citric acid and 1.57 g of boric acid in the flask. Add more water to make a final volume of 250 mL. Place 20 mL of this stock solution into a 250-mL Erlenmeyer flask. Titrate the solution to pH 6.5 by adding 0.1 *N* HCl. When the solution is pH 6.5, analytically transfer this solution to a 100-mL volumetric flask and add more water to make a final volume of 100 mL.

8.5.4 *p-Nitrophenyl Phosphate (PNP, 5 mM)*—Add 80 mL of MUB buffer to a 100-mL volumetric flask and dissolve 0.1675 g of disodium-*p*-nitrophenyl phosphate tetrahydrate in the buffer. Add more MUB buffer to the flask to make a final volume of 100 mL. Store the solution in refrigerator.

8.5.5 *Sodium hydroxide (0.5 M)*—In a 1-L volumetric flask, dissolve 20.0 g of NaOH (mol wt 40.0) in 200 mL of double distilled water and dilute the solution with more water to make a final volume of 1 L.

8.5.6 *filter paper*—Whatman No. 12 and Whatman No. 2v, folded.

8.6 *Method 05.04-A2 Phosphomonoesterase (alkaline phosphatase):*

8.6.1 *THAM-sulfuric buffer (0.05 M, pH 8.0)*—In a 1-L volumetric flask, dissolve 6.1 g of tris (hydroxymethyl) aminomethane (THAM) in 800 mL of double distilled water. Adjust the pH to 8.0 by titration with calibrated 0.2 N H_2SO_4 and dilute the solution with water to make a volume of 1 L.

8.6.2 *p-Nitrophenyl Phosphate (PNP, 5 mM)*—Add 80 mL of THAM buffer to a 100-mL volumetric flask and dissolve 0.1811 g of sodium *p*-nitrophenyl phosphate in the buffer. Add more THAM buffer to the flask to make a final volume of 100 mL. Store the solution in refrigerator.

8.6.3 *THAM-Sodium Hydroxide Extractant Solution (0.1 M THAM, pH 12)*—In a 1-L volumetric flask, dissolve 12.2 g of THAM in 800 mL of double distilled water, adjust the pH to 12 by titration with 0.5 M NaOH. Add more water to make a final volume of 1 L.

8.6.4 *THAM Diluent (0.1 M, pH 10)*—In a 1-L volumetric flask, dissolve 12.2 g of THAM in 800 mL of double distilled water. Add more water to make a final volume of 1 L.

8.7 *Method 05.04-A3 Phosphodiesterase:*

8.7.1 *THAM-sulfuric buffer (0.05 M, pH 8.0)*—In a 1-L volumetric flask, dissolve 6.1 g of tris (hydroxymethyl) aminomethane (THAM) in 800 mL of double distilled water. Adjust the pH to 8.0 by titration with calibrated 0.2 *N* H_2SO_4 and dilute the solution with water to make a volume of 1 L.

8.7.2 *Bis-p-Nitrophenyl Phosphate (BPNP, 5 mM)*—Add 80 mL of THAM buffer to a 100-mL volumetric flask and dissolve 0.1811 g of sodium bis-*p*-nitrophenyl phosphate in the buffer. Add more THAM buffer to the flask to make a final volume of 100 mL. Store the solution in refrigerator.

8.7.3 *THAM-Sodium Hydroxide Extractant Solution (0.1M THAM, pH 12)*—In a 1-L volumetric flask, dissolve 12.2 g of THAM in 800 mL of double distilled water, adjust the pH to 12 by titration with 0.5 M NaOH. Add more water to make a final volume of 1 L.

8.7.4 *THAM Diluent (0.1 M, pH 10)*—In a 1-L volumetric flask, dissolve 12.2 g of THAM in 800 mL of double distilled water. Add more water to make a final volume of 1 L.

9. Calibration of the Apparatus for Method A

9.1 *Method 05.04-A1 Phosphomonoesterase (acid phosphatase):*

9.1.1 Transfer an aliquot of water into a colorimeter tube and measure the absorbance at 400 nm. This is the absorbance measured at 0 µg of PN mL^{-1}.

9.1.2 Place an aliquot of the 10 µg of PN mL^{-1} into a colorimeter tube and measure the absorbance.

9.1.3 Repeat 9.1.2 for the 20, 30, 40 and 50 µg of PN mL^{-1} standards.

9.1.4 Determine the calibration curves. Plot the absorbance (y-axis, dependent variable) versus the concentration of PN in solution (x-axis, independent variable). Fit a linear calibration line.

9.2 *Method 05.04-A2 Phosphomonoesterase (alkaline phosphatase):*

9.2.1 Transfer an aliquot of the THAM diluent into a colorimeter tube and measure the absorbance at 400 nm. This is the absorbance measured at 0 µg of PN mL^{-1} .

9.2.2 Place an aliquot of the 10 µg of PN mL^{-1} into a colorimeter tube and measure the absorbance.

9.2.3 Repeat 9.2.2 for the 20, 30, 40 and 50 µg of PN mL^{-1} standards.

9.2.4 Determine the calibration curves. Plot the absorbance (y-axis, dependent variable) versus the concentration of PN in solution (x-axis, independent variable). Fit a linear calibration line.

9.3 *Method 05.04-A3 Phosphodiesterase:*

9.3.1 Transfer an aliquot of the THAM diluent into a colorimeter tube and measure the absorbance at 400 nm. This is the absorbance measured at 0 µg of PN mL^{-1} .

9.3.2 Place an aliquot of the 10 µg of PN mL^{-1} into a colorimeter tube and measure the absorbance.

9.3.3 Repeat 9.3.2 for the 20, 30, 40 and 50 µg of PN mL^{-1} standards.

9.3.4 Determine the calibration curves. Plot the absorbance (y-axis, dependent variable) versus the concentration of PN in solution (x-axis, independent variable). Fit a linear calibration line.

10. Procedure for Method A

10.1 *Method 05.04-A1 Phosphomonoesterase (acid phosphatase):*

10.1.1 Place 1 g of compost into two 50-mL Erlenmeyer flask. Label flasks as Compost and Control. Label a third flask as Blank - this is the sample without compost.

10.1.2 Add 0.2 mL of toluene and 4 mL of MUB buffer and 1 mL of *p*-Nitrophenyl phosphate (PNP) to the Compost sample and Blank flasks. Add 0.2 mL of toluene and 4 mL of MUB buffer to the Control sample.

10.1.3 Swirl flasks for 30 s to mix contents.

10.1.4 Stopper the flasks and incubate at 37°C for 1 h.

10.1.4.1 After incubation, stop phosphatase activity by cooling sample to 2°C for 20 min.

10.1.5 Remove stoppers and add 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH to flasks. Swirl flasks for 30 s to mix contents.

10.1.6 Filter the suspension in the Compost sample flask through a Whatman No. 12 folded filter paper.

10.1.7 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 400 nm.

10.1.8 Calculate the *p*-Nitrophenol (PN) content of the filtrate by reference to the calibration curve plotted with PN standards.

10.1.9 Add 1 mL of PNP solution to Control flask and immediately filter the suspension through a Whatman No. 12 folded filter paper.

10.1.10 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 400 nm.

10.1.11 Calculate the PN content of the control filtrate by reference to the calibration curve plotted with PN standards.

10.1.12 Filter the suspension in the Blank sample flask through a Whatman No. 12 folded filter paper.

10.1.13 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 400 nm.

10.1.14 Calculate the *p*-Nitrophenol (PN) content of the filtrate in the Blank sample with reference to the calibration curve plotted with PN standards.

10.2 *Method 05.04-A2 Phosphomonoesterase (alkaline phosphatase):*

10.2.1 Place 1 g of compost into two 50-mL Erlenmeyer flask. Label flasks as Control and Sample. Label a third flask as Blank - this is the sample without compost.

10.2.2 Add 0.2 mL of toluene and 4 mL of THAM buffer pH 8.0 and 1 mL of *p*-Nitrophenyl phosphate (PNP) to the Compost sample and Blank flasks. Add 0.2 mL of toluene and 4 mL of THAM buffer to the Control sample.

10.2.3 Swirl flasks for 30 s to mix contents.

10.2.4 Stopper the flasks and incubate at 37°C for 1 h.

10.2.4.1 After incubation, stop phosphatase activity by cooling sample to 2°C for 20 min.

10.2.5 Remove stoppers and add 1 mL of 0.5 M $CaCl_2$ and 4 mL of THAM-NaOH extractant solution to flasks. Swirl flasks for 30 s to mix contents.

10.2.6 Filter the suspension in the Compost sample flask through a Whatman No. 2 v-folded filter paper.

10.2.7 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 400 nm.

10.2.8 Calculate the *p*-Nitrophenol (PN) content of the filtrate by reference to the calibration curve plotted with PN standards.

10.2.9 Add 1 mL of PNP solution to Control flask and immediately filter the suspension through a Whatman No. 2 v-folded filter paper.

10.2.10 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 400 nm.

10.2.11 Calculate the PN content of the control filtrate by reference to the calibration curve plotted with PN standards.

10.2.12 Filter the suspension in the Blank sample flask through a Whatman No. 2 v-folded filter paper.

10.2.13 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 400 nm.

10.2.14 Calculate the *p*-Nitrophenol (PN) content of the filtrate in the Blank sample with reference to the calibration curve plotted with PN standards.

10.3 *Method 05.04-A3 Phosphodiesterase (for use with alkaline composts only):*

10.3.1 Place 1 g of compost into two 50-mL Erlenmeyer flask. Label flasks as Control and Compost. Label a third flask as Blank - this is the sample without compost.

10.3.2 Add 0.2 mL of toluene and 4 mL of THAM buffer and 1 mL of Bis-*p*-Nitrophenyl Phosphate (BPNP) to the Compost sample and Blank flasks. Add 0.2 mL of toluene and 4 mL of THAM buffer to the Control sample.

10.3.3 Swirl flasks for 30 s to mix contents.

10.3.4 Stopper the flasks and incubate at 37°C for 1 h.

10.3.4.1 After incubation, stop phosphatase activity by cooling sample to 2°C for 20 min.

10.3.5 Remove stoppers and add 1 mL of 0.5 M $CaCl_2$ and 4 mL of THAM-NaOH extractant solution to flasks. Swirl flasks for 30 s to mix contents.

10.3.6 Filter the suspension in the Compost sample flask through a Whatman No. 2 v-folded filter paper.

10.3.7 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 400 nm.

10.3.8 Calculate the *p*-Nitrophenol (PN) content of the filtrate by reference to the calibration curve plotted with PN standards.

10.3.9 Add 1 mL of BPNP solution to Control flask and immediately filter the suspension through a Whatman No. 2 v-folded filter paper.

10.3.10 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 400 nm.

10.3.11 Calculate the PN content of the control filtrate by reference to the calibration curve plotted with PN standards.

10.3.12 Filter the suspension in the Blank sample flask through a Whatman No. 2 v-folded filter paper.

10.3.13 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 400 nm.

10.3.14 Calculate the *p*-Nitrophenol (PN) content of the filtrate in the Blank sample with reference to the calibration curve plotted with PN standards.

11. Calculations for Method A

11.1 *Method 05.04-A Phosphomonoesterase (acid phosphatase), Phosphomonoesterase (alkaline phosphatase), and Phosphodiesterase:*

11.1.1 Fit linear relationship to calibration curve and determine constants B and D with units mL μg^{-1} and unitless, respectively:

$$A = B \times St + D \qquad \textit{Equation 11.1.1}$$

11.1.2 Calculate concentration of PN μg mL^{-1} in Compost filtrate:

$$St = (A - D) \div B \qquad \textit{Equation 11.1.2}$$

11.1.3 Calculate concentration of PN μg mL^{-1} in Control filtrate:

$$C = (A - D) \div B \qquad \textit{Equation 11.1.3}$$

11.1.4 Calculate concentration of PN μg mL^{-1} in Blank filtrate:

$$BF = (A - D) \div B \qquad \textit{Equation 11.1.4}$$

11.1.5 Calculate concentration of PN μg in compost sample filtrate as a result of catalysis of phosphate substrate by phosphomonoesterase:

$$E = (St - C - BF) \times 10.2 \qquad \textit{Equation 11.1.5}$$

11.1.6 Calculate activity for each enzyme (μg of *p*-nitrophenol released g^{-1} of air dried compost h^{-1}):

$$AC = E \div 1 \times M \qquad \textit{Equation 11.1.6}$$

where:

A = absorbance of *p*-nitrophenol in sample, standard or blank (unitless),
B = slope of fitted curve (1/μg mL^{-1}, or mL μg^{-1}),
C = concentration of *p*-nitrophenol (μg mL^{-1}),
D = intercept of fitted curve (unitless),
St = μg of *p*-nitrophenol mL^{-1} in standard filtrate,
C = μg of *p* -nitrophenol mL^{-1} in Control sample filtrate,
BF = μg of *p* -nitrophenol mL^{-1} in Blank sample filtrate,
E = μg of *p* -nitrophenol mL^{-1} in sample filtrate as a result of catalysis of phosphate substrate by phosphomonoesterase (acid phosphatase),
10.2 = volume of solution in flasks (mL),
AC = phosphomonoesterase activity (acid phosphatase, μg of PN g^{-1} of air dried compost h^{-1}), and
M = ratio of oven dried sample weight versus as-received weight, unitless.

Test Method: Enzyme Activity and Analysis. Dehydrogenases						**Units:** mg g^{-1} dw 24 h^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
			05.04-B	05.04-B		05.04-B		05.04-B

05.04-B DEHYDROGENASES

CAUTION—This method was developed for the determination of dehydrogenase activity in soils and may provide unpredictable results when applied to composts and other materials high in water-soluble organic matter.

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12. Apparatus for Method B

12.1 *Spectrophotometer*—with facility to measure the absorbance at 485 nm.

12.2 *Volumetric Flasks*—25-mL and 100-mL.

12.3 *Erlenmeyer Flasks*—25-mL with stoppers.

12.4 *Colorimeter Tubes*—two per experiment.

13. Reagents and Materials for Method B

13.1 *Methanol*—reagent grade.

13.2 *Water*—double distilled.

13.3 *2, 3, 5-triphenyl-tetrazolium chloride (TTC, 3%)*—Place 100 mL of water into a 100-mL volumetric flask. Dissolve 3 g of 2, 3, 5-triphenyl-tetrazolium chloride in the water. Add more water to a final volume of 100 mL.

NOTE 1B—Store in the dark; this solution turns yellow when exposed to light.

13.4 *Triphenyl formazan standard solution*—Place 50 mL of methanol into a 100 mL volumetric flask. Dissolve 2.5 g of triphenyl formazan in the methanol. Add more methanol to a final volume of 100 mL. This is 25 mg mL^{-1} standard triphenyl formazan solution.

13.5 *Standard triphenyl formazan solutions containing 20, 10 and 5 and 0.5 mg of triphenyl formazan* mL^{-1}—Place 20, 10, 5 and 0.5 mL of standard triphenyl formazan solution into 25-mL volumetric flasks, respectively. To each flask add sufficient methanol to make a final volume of 25 mL.

13.6 *filter*—glass fiber.

14. Calibration of Apparatus for Method B

14.1 Transfer an aliquot of the methanol diluent into a colorimeter tube and measure the absorbance at 485 nm. This is the absorbance measured at 0 mg of triphenyl formazan mL^{-1}.

14.2 Place an aliquot of the 0.5 mg of triphenyl formazan mL^{-1} into a colorimeter tube and measure the adsorbence at 485 nm.

14.3 Repeat 14.2 for the 1, 5, 10 and 20 mg of triphenyl formazan mL^{-1} standards.

14.4 Determine the calibration curves. Plot the absorbance (y-axis, dependent variable) versus the concentration of triphenyl formazan in solution (x-axis, independent variable). Fit a linear calibration line.

15. Procedure for Method B

15.1 Place 3 g of compost into each of two 25-mL Erlenmeyer flasks. Label flasks as Control and Sample. Label a third 25-mL Erlenmeyer flask Compost Blank - this is the control without compost.

15.2 Add 3 mL of water and 3 mL of 3% TCC to the Compost sample and Blank sample flasks only. Add 6 mL of water to the Control sample flask.

15.3 Swirl all flasks for 30 s to mix contents.

15.4 Stopper the flasks and incubate at 37°C for 24 h in the dark.

15.5 Remove stoppers and add 10 mL of methanol to the three flasks. Swirl flasks for 5 min.

15.6 Filter the suspension in the Compost sample flask through a glass fiber filter into a 100-mL volumetric flask.

15.7 Wash the glass fiber filter with methanol until the reddish color caused by the reduced triphenyl-tetrazoliumchloride disappears and collect all filtrate.

15.8 Add methanol to the collection volumetric flask to a final volume of 100 mL.

15.9 Transfer an aliquot of the filtrate into a colorimeter tube and measure the absorbance at 485 nm.

15.10 Calculate the triphenyl formazan content of the filtrate by reference to the calibration curve plotted with triphenyl formazan standards.

15.11 Repeat steps 15.5 through 15.9 for the Control sample and Blank sample.

16. Calculations for Method B

16.1 Fit linear relationship to calibration curve and determine constants B and D with units $1/\mu g\ mL^{-1}$ and unitless, respectively:

$$A = B \times St + D \qquad \textit{Equation 16.1}$$

16.2 Calculate concentration of TPF mg mL^{-1} in Sample filtrate:

$$St = (A - D) \div B \qquad \textit{Equation 16.2}$$

16.3 Calculate concentration of TPF mg mL^{-1} in Control filtrate:

$$C = (A - D) \div B \qquad \textit{Equation 16.3}$$

16.4 Calculate concentration of TPF mg mL^{-1} in Compost Blank filtrate:

$$CB = (A - D) \div B \qquad \textit{Equation 16.4}$$

16.5 Calculate mg of TPF in sample filtrate as a result of catalysis of TTC by dehydrogenases:

$$E = (St - C - CB) \times 100 \qquad \textit{Equation 16.5}$$

16.6 Calculate activity of dehyrogenase AC (mg of TF released g^{-1} of air dried compost 24 h^{-1}):

$$AC = E \div 3 \times M \qquad \textit{Equation 16.6}$$

where:

A = absorbance of triphenyl formazan in sample, standard or blank, unitless,

B = slope of fitted curve, $1 \div$ mg mL^{-1},

C = concentration of triphenyl formazan, mg mL^{-1},

D = intercept of fitted curve, unitless,

St = mg of triphenyl formazan mL^{-1} in standard filtrate,

C = mg of triphenyl formazan mL^{-1} in Control filtrate,

CB = mg of triphenyl formazan mL^{-1} in Blank filtrate,

E = mg of triphenyl formazan in sample filtrate as a result of catalysis of triphenyl-tetrazolium chloride by dehydrogenase,

100 = final volume of samples extracted with methanol from Compost, Control and Blank samples, mL,

AC = activity of dehyrogenase, mg of TPF g^{-1} of air dried compost 24 h^{-1}, and

M = ratio of oven dried sample weight versus as-received weight, unitless.

Test Method: Enzyme Activity and Analysis. Proteases						**Units:** μmoles g^{-1} dw h^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.04-C	05.04-C	05.04-C		05.04-C		05.04-C

05.04-C PROTEASES

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17. Apparatus for Method C

17.1 *Spectrophotometer*—with facility to measure the absorbance at 570 nm.

17.2 *Volumetric Flasks*—250-mL and 500-mL.

17.3 *Beakers*—250-mL.

17.4 *Colorimeter Tubes*—two per experiment.

17.5 *Vial*—5-mL, glass, with stopper.

17.6 *Centrifuge tubes*—50-mL.

17.7 *Centrifuge*—set for 8000 *g* with timer at 10 min.

17.8 *Water bath*

18. Reagents and Materials for Method C

18.1 *Tris(hydroxymethyl)aminomethane (THAM)-HCl buffer (0.1 M, pH 8.0)*—Dissolve 12.2 g of THAM in 800 mL of H_2O. Adjust solution pH to 8.0 by titrating with 0.1 *M* HCl. Dilute to 1 L with H_2O.

18.2 *Benzyloxycarbonyl-phenylalanyl-leucine solution*—2 m*M*.

18.3 *HCl (5 M)*—Reagent grade concentrated HCl contains close to 38% HCl. Place 50 mL of water into a 250-mL beaker and add 41.5 mL of concentrated HCl. Add more water to a final volume of 100 mL.

18.4 *NaOH (1 M)*—Place 50 mL of water into a 250-mL beaker and dissolve 4 g of NaOH to the beaker. Add more water to a final, volume of 100 mL.

18.5 *Sodium Acetate Buffer (pH 5.5)*—Add 200 mL of water to 500-mL volumetric flask. Dissolve 555 g of $NaOAc{\cdot}3H_2O$ in the water. Stir in a steam or water bath until solution is complete. Cool to room temperature, add 125 mL of glacial acetic acid. Add water to a final volume of 500 mL. The solution should be about pH 5.5. If final adjustment of the pH is necessary add drops of concentrated NaOH. Store the buffer at 4°C.

18.6 *Ninhydrin Reagent Solution*—Add 75 mL of monomethyl ether of ethylene glycol into a 250-mL beaker. Dissolve 2 g of ninhydrin ($C_9H_6O_4$ mol wt 178.14) , 0.3 g of hydrindantin ($C_{18}H_{10}O_6$ mol wt: 322.27) in the solvent. Add sodium acetate buffer to a final volume of 100 mL. Care should be taken to avoid air bubbles. Transfer solution to a dark storage glass bottle and store the solution under nitrogen. Refer to Moore and Stein, 1954, J. Biol. Chem. 211:907-913.

18.7 *Leucine Standard Solution (500 μmoles L^{1})*—Add 50 mL of acetate buffer into a 100-mL flask. Dissolve 0.00655 g of leucine in the buffer. Add more acetate buffer to a final volume of 100 mL.

18.7.1 *Standard leucine solutions containing 40, 30, 20, 10, 5 and 2 μmoles leucine L^{-1}*—Add 20, 15, 10, 5, 2.5 and 1 mL of the 500 μmoles of leucine L^{-1} solution to six 250-mL volumetric flasks, respectively. Add sodium acetate buffer to a final volume of 250 mL.

19. Calibration of Apparatus for Method C

19.1 Add 1 mL of 2 μmoles leucine L^{-1} to a glass vial and add 1 mL of ninhydrin reagent to it. Stopper the vial and shake the contents for < 10 s and heat in a water bath at 100°C for 15 min. Cool the solution to less than 30°C and transfer an aliquot of the solution to a colorimeter tube and measure the absorbance at 570 nm.

19.2 Repeat step 19.1 for 5, 10, 20, 30 and 40 μmoles leucine L^{-1}; replace the leucine solution with 1 mL of acetate buffer when analyzing the control sample.

19.3 Determine the calibration curves. Plot the absorbance (y-axis, dependent variable) versus the concentration of leucine in solution (x-axis, independent variable). Fit a linear calibration line.

20. Procedure for Method C

20.1 Place 3 g of compost into two 50-mL centrifuge tubes. Label tubes as Compost and Control. Label a third 50-mL centrifuge tube Blank - this is the control without compost.

20.2 Add 10.8 mL of 0.1 M Tris-HCl buffer, pH 8.0 and 12 mL of 2 mM of benzyloxycarbonyl-phenylalanyl-leucine solution to the Compost and Blank sample tubes only. Add 22.8 mL of 0.1 M Tris-HCl buffer to the Control sample tube.

20.3 Stopper the tubes and agitate and incubate them at 40°C for 1 h.

20.4 Cool the tubes rapidly to 20°C and add 1.2 mL of 5 *N* HCl to each flasks. This should cause enzyme activity to cease.

20.5 Centrifuge the Compost, Control and Blank tubes at 8000 *g* for 10 min.

20.6 Decant 0.8 mL of the supernatant from the Compost sample into a vial and neutralize it with 0.2 mL of 1 *N* NaOH. Measure the pH of the mixture and add a few drops of either 1 *N* NaOH or HCl so that it lies between pH 4 and pH 6. Repeat this procedure for the Control and Blank samples.

20.7 Add 1 mL of the ninhydrin solution to the neutralized supernatant. Stopper the tubes and mix the contents briefly (10 s) and heat the samples in a water bath for 15 min. Cool the tubes to below 30°C.

20.8 Transfer an aliquot of the ninhydrin treated Compost sample into a colorimeter tube and measure the absorbance at 570 nm.

20.9 Calculate the amino acid content of the filtrate by reference to the calibration curve plotted with leucine standards.

20.10 Repeat steps 20.7 through 20.9 for the Control and Blank samples.

21. Calculations for Method C

21.1 Fit linear relationship to calibration curve and determine constants B and D with units 1/μmoles of leucine mL^{-1} and unitless, respectively:

$$A = B \times St + D \qquad \textit{Equation 21.1}$$

21.2 Calculate concentration of amino acids μmoles of leucine mL^{-1} in compost:

$$St = (A - D) \div B \qquad \textit{Equation 21.2}$$

21.3 Calculate concentration of amino acids μmoles of leucine mL^{-1} in control filtrate:

$$C = (A - D) \div B \qquad \textit{Equation 21.3}$$

21.4 Calculate concentration of amino acids μmoles of leucine mL^{-1} in Blank filtrate:

$$CB = (A - D) \div B \qquad \textit{Equation 21.4}$$

21.5 Calculate μmoles of amino acids in compost as a result of catalysis of benzyloxycarbonyl-phenylalanyl-leucine by proteaseses:

$$E = (St - C - CB) \times 24 \qquad \textit{Equation 21.5}$$

21.6 Calculate activity of protease (μmoles of leucine released g^{-1} of air dried compost h^{-1}):

$$AC = E \div 3 \times M \qquad \textit{Equation 21.6}$$

where:

A = absorbance of amino acid-ninhydrin reagent in standard, blank or sample, unitless,
B = slope of fitted curve, 1 ÷ μmoles mL^{-1},
C = concentration of leucine, μmoles mL^{-1},
D = intercept of fitted curve, unitless,
St = μmoles of leucine mL^{-1} in standard filtrate,
C = μmoles of leucine mL^{-1} in Control filtrate,
CB = μmoles of leucine mL^{-1} in Blank filtrate,
E = μmoles of leucine mL^{-1} in sample filtrate as a result of catalysis of leucine by protease,
24 = volume of solution, mL,
3 = mass of compost, g
AC = activity of protease, μmoles of leucine g^{-1} of air dried compost h^{-1},
M = ratio of oven dried sample weight versus as-received weight, unitless.

Test Method: Enzyme Activity and Analysis. Cellulases						**Units:** mg g^{-1} dw 24 h^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.04-D	05.04-D	05.04-D		05.04-D		05.04-D

05.04-D CELLULASES

CONTRIBUTED BY—Dr. Brenda F. Farrell, Edaphos International

22. Apparatus for Method D

22.1 *Spectrophotometer*—with facility to measure the absorbance at 625 nm.

22.2 *Volumetric Flasks*—25-mL, 100-mL, 250-mL and 1-L.

22.3 *Cuvettes*—two per experiment.

22.4 *Vial*—5-mL, glass, with stopper.

22.5 *Test Tubes*—10-mL.

22.6 *Centrifuge Tubes*—50-mL.

22.7 *Pipette*—10 mL, TD.

22.8 *Rotary Shaker*—equipped with timer set at 24 h and bath set at 30°C.

22.9 *Centrifuge*—set for 17000 *g* with timer at 10 min.

23. Reagents and Materials for Method D

23.1 *Acetate buffer (0.2 M, pH 5.9, 1 L)*—Add 500 mL of deionized distilled water to a 1-L volumetric flask and add 16.4 g of sodium acetate and 1.33 g of acetic acid to the flask. Add more water to a final volume of 1 L.

23.2 *Sulfuric acid (95% v/v)*—Reagent grade sulfuric acid contains 93-98% H_2SO_4. Use as received.

23.3 *Carboxymethyl cellulose in acetate buffer (1%)*—Add 100 mL of acetate buffer into a 250-mL volumetric flask and add 2.5 g of carboxymethyl cellulose. Add more buffer to a final volume of 250 mL. The carboxymethyl cellulose is available at various viscosities. Use the polymer of lowest viscosity a 1% solution in water is about 5 centipoise.

23.4 *Anthrone in sulfuric acid (0.2% solution)*—Add 50 mL of concentrated sulfuric acid into a 100-mL volumetric flask and add 0.2 g of anthrone ($C_{14}H_{10}O$, mol wt: 194.23). Add more acid to a final volume of 100 mL.

CAUTION—This reagent should be made up in a fume hood.

23.5 *Standard glucose solution*—Place 50 mL of 0.2 M acetate buffer into a 100-mL volumetric flask. Dissolve 2.5 g of glucose to this flask. Add more buffer to a final volume of 100 mL. This is 25 mg mL^{-1} standard glucose solution. Store in freezer at -4°C.

23.5.1 *Standard glucose solutions containing 20, 15, 10 and 5 mg of glucose mL^{-1}*—Place 20, 15, 10 and 5 mL of standard glucose solution into 25-mL volumetric flasks, respectively. To each flask add sufficient acetate buffer to make a final volume of 25 mL and mix contents. Store in freezer.

NOTE 1D—Do not store solutions at room temperature.

24. Calibration of Apparatus for Method D

24.1 Transfer 5 mL of 0.2 M acetate buffer into test tube and rapidly pipette 10 mL of anthrone solution. Mix contents. After 15 min of incubation transfer an aliquot of this solution to a spectrophotometer tube and measure the absorbance at 625 nm. This is the absorbance measured at 0 mg of glucose mL^{-1}.

24.2 Transfer 5 mL of 5 mg mL^{-1} glucose solution into a test tube and rapidly pipette 10 mL of anthrone solution. Mix contents. After 15 min incubation transfer and aliquot of this solution to a spectrophotometer cuvette and measure the absorbance at 625 nm. This is the absorbance measured at 5 mg of glucose mL^{-1}.

24.3 Repeat 24.2 for the 10, 15, 20 and 25 mg of glucose mL^{-1} standards.

24.4 Determine the calibration curves. Plot the absorbance (y-axis, dependent variable) versus the concentration of glucose in solution (x-axis, independent variable). Fit a linear calibration line.

25. Procedure for Method D

25.1 Place 3 g of compost into two 50-mL centrifuge tubes. Label tubes as Control and Compost. Label a third 50-mL centrifuge tube Blank - this is the control without compost.

25.2 Add 10 mL of 0.2 M acetate buffer (pH 5.9), and 10 mL of 1 % carboxymethyl cellulose to the Compost and Blank tubes only. Add 20 mL of 0.2 M acetate buffer (pH 5.9) to the Control tubes.

25.3 Stopper the tubes and agitate on a rotary shaker for 24 h at 30°C.

25.4 Centrifuge the tubes at 17000 *g* for 10 min.

25.5 Place 5 mL of the supernatant from the Compost sample into a glass test tube and rapidly pipette 10 mL of anthrone solution into this glass test tube. Shake test tube for 30 s to mix contents and incubate at room temperature for 15 min.

25.6 Transfer an aliquot of the anthrone treated Compost sample to a spectrophotometer cuvette and measure the absorbance at 625 nm.

25.7 Calculate the reducing sugar content of the filtrate by reference to the calibration curve plotted with glucose standards.

25.8 Repeat steps 25.5 through 25.7 for the sample found in the Control and Blank tubes.

26. Calculations for Method D

26.1 Fit linear relationship to calibration curve and determine constants B and D with units $1 \div \text{mg mL}^{-1}$ and unitless, respectively:

$$A = B \times St + D \qquad \textit{Equation 26.1}$$

26.2 Calculate concentration of glucose mg mL^{-1} in the Compost sample:

$$St = (A - D) \div B \qquad \textit{Equation 26.2}$$

26.3 Calculate concentration of glucose mg mL^{-1} in the Control sample:

$$C = (A - D) \div B \qquad \textit{Equation 26.3}$$

26.4 Calculate concentration of glucose mg mL^{-1} in the Blank sample:

$$CB = (A - D) \div B \qquad \textit{Equation 26.4}$$

26.5 Calculate amount of glucose (mg) in compost as a result of catalysis of carboxymethyl cellulose by cellulases:

$$E = (S - C - CB) \times 20 \qquad \textit{Equation 26.5}$$

26.6 Calculate activity of cellulase (mg of glucose released g^{-1} of air dried compost 24 h^{-1}):

$$AC = E \div 3 \times M \qquad \textit{Equation 26.6}$$

where:

A = absorbance of glucose -anthrone solution, unitless,
B = slope of fitted curve, $1 \div \text{mg mL}^{-1}$,
C = concentration of glucose, mg mL^{-1},
D = intercept of fitted curve, unitless,
St = mg of glucose mL^{-1} in standard filtrate,
C = mg of glucose mL^{-1} in Control sample,
CB = mg of glucose mL^{-1} in Blank sample,
E = mg of glucose in compost supernatant as a result of catalysis of carboxymethyl cellulose by cellulase,
20 = volume of solution, mL,
3 = mass of compost, g
AC = activity of cellulase, mg of glucose g^{-1} of air dried compost 24 h^{-1},
M = ratio of oven dried sample weight versus as-received weight, unitless.

Test Method: Enzyme Activity and Analysis. Peroxidases						**Units:** μmoles g^{-1} dw 30 sec^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.04-E	05.04-E	05.04-E		05.04-E		05.04-E

05.04-E PEROXIDASES

CONTRIBUTED BY—Dr. Brenda F. Farrell, Edaphos International

27. Apparatus for Method E

27.1 *Spectrophotometer*—with facility to measure the absorbance at 460 nm.

27.2 *Volumetric Flasks*—10-mL, 50-mL, 100-mL and 1-L.

27.3 *Cuvettes*—two per experiment.

27.4 *Vial*—5-mL, glass, with stopper.

27.5 *Test Tubes*—10-mL.

27.6 *Centrifuge Tubes*—50-mL.

27.7 *Pipette*—10 mL, TD.

27.8 *Rotary Shaker*—equipped with timer set at 24 h and bath set at 30°C.

27.9 *Centrifuge*—set for 8000 *g* with timer at 10 min.

28. Reagents and Materials for Method E

28.1 *Phosphate buffer (0.2 M, pH 6.0) 1 L*—Place 500 mL of water in 1-L volumetric flask. Dissolve 20.86 g of NaH_2PO_4 (0.173 M) and 4.6853 g of $KHPO_4$ (0.0265 M). Add more water to a final volume of 1 L. Measure the pH of the solution with glass electrode and adjust to pH 6.0 by adding drops of either 5 *N* HCl or 5 *N* NaOH.

28.2 *H_2O_2 (0.06%) in 0.2 M Phosphate buffer*—Place 50 mL of phosphate buffer in 100-mL volumetric flask. Dissolve 0.00204 g of H_2O_2 in the buffer. Add more buffer to a final volume of 100 mL. H_2O_2 is a strong oxidizing agent, the reagent should be made up in a fume hood.

28.3 *o-dianisidine in methanol (0.05%)*—Place 25 mL of methanol into a 50-mL volumetric flask. Dissolve 0.25 g of o-dianisidine in the methanol (mol. wt. of o-dianisidine is 244.29). Add more methanol to a final volume of 50 mL.

28.4 *filters*—0. 22 μm Millipore.

28.5 *Horseradish peroxidase solution (purified, 1 mg mL^{-1})*—Place 25 mL of phosphate buffer in 50-mL volumetric flask. Dissolve 50 mg of horseradish peroxidase in the buffer. Add more buffer to final volume of 50 mL. Store in freezer at -4°C.

28.5.1 *Solutions containing 100, 10 and 1 μg mL^{-1} of horseradish peroxidase in phosphate buffer*—Transfer 1.00, 0.10 and 0.01 mL of the 1 mg mL^{-1} horseradish peroxidase solution into three 10-mL volumetric flasks, respectively. Add more buffer to a final volume of 10 mL.

29. Calibration of Apparatus for Method E

29.1 Place 2.7 mL of the 1 μg mL^{-1} of horseradish peroxidase solution in a spectrometer cuvette. Add 0.05 mL of the 0.5% o-dianisidine in methanol solution and 0.3 mL of the 0.06% H_2O_2 to the cuvette. Mix the contents.

29.2 Measure the increase in the absorbance at 460 nm every 30 s for 5 min.

29.3 Repeat steps 29.1 and 29.2 for the 10 and 100 μg mL^{-1} of horseradish peroxidase solutions.

29.4 Plot the absorbance versus time and check that the relationship is linear over the 5 min period.

30. Procedure for Method E

30.1 Place 9 g of compost into two 50-mL centrifuge tubes. Label tubes as Control and Compost. Label a third 50-mL centrifuge tube Blank - this is the control without compost.

30.2 Add 25 mL of phosphate buffer to the three centrifuge tubes and agitate samples for 5 min on a rotary shaker.

30.3 Centrifuge the tubes for 10 min at 8000 *g* and decant the supernatant.

30.4 The supernatant from the Control, Compost and Blank tubes is sterilized by filtering through a 0.22 μm filter. The extracts are handled aseptically until tested.

30.5 Place 2.7 mL of the sterile supernatant from the Compost sample in a spectrometer cuvette. Add 0.05 mL of the 0.5% o-dianisidine in methanol solution and 0.3 mL of the 0.06% H_2O_2 to the cuvette. Mix the contents.

30.6 Measure the increase in the absorbance at 460 nm every 30 s for 5 min. Calculate the H_2O_2 decomposed every 30 s by use of the molar absorbency of o-dianisidine at 1.13×10^4 $cm^{-1}M^{-1}$.

30.7 Place 2.7 mL of the sterile supernatant from the Control sample in a spectrometer cuvette. Add 0.35 mL of the 0.06% H_2O_2 to the cuvette. Mix the contents.

30.8 Repeat step 30.6.

30.9 Place 2.7 mL of the sterile supernatant from the Blank sample in a spectrometer cuvette. Add 0.05 mL of the 0.5% o-dianisidine in methanol solution and 0.3 mL of the 0.06% H_2O_2 to the cuvette. Mix the contents.

30.10 Repeat step 30.6.

31. Calculations for Method E

31.1 The absorbance of o-dianisidine at 460 nm is related to the concentration of o-dianisidine by the following relationship:

$$A_{460} = \varepsilon \times C \times L \qquad \textit{Equation 31.1}$$

31.2 Concentration of oxidized o-dianisidine (M) in the Compost sample solution at 30 s:

$$C = \Delta A \div (\varepsilon \times L) \qquad \textit{Equation 31.2}$$

31.3 Concentration of oxidized o-dianisidine (M) in the Control sample solution at 30 s:

$$CC = \Delta AC \div (\varepsilon \times L) \qquad \textit{Equation 31.3}$$

31.4 Concentration of oxidized o-dianisidine (M) in the Blank sample solution at 30 s:

$$CB = \Delta AB \div (\varepsilon \times L) \qquad \textit{Equation 31.4}$$

31.5 Concentration (M) of oxidized o-dianisidine (M) in the compost sample as a result of catalysis of o-dianisidine by peroxidase at 30 s:

$$CN = (C - CC - CB) \qquad \textit{Equation 31.5}$$

31.6 μM of oxidized o-dianisidine in the compost as a result of peroxidase activity at 30 s:

$$D = (CN \div 1000 \times 25) \times 1.1296 \times 1{\times}10^6 \qquad \textit{Equation 31.6}$$

31.7 Calculate activity of peroxidase (μM of H_2O_2 decomposed g^{-1} of air dried compost 30 sec^{-1})

$$H = D \div 9 \times M \qquad \textit{Equation 31.7}$$

where:

A = absorbance of oxidized o-dianisidine at 460 nm,

ε = extinction coefficient of oxidized o-dianisidine at 1.31×10^4 $cm^{-1}M^{-1}$,

L = path length, cm,

ΔA = change in the absorbance of the oxidized o-dianisidine in Compost sample,

ΔAB = change in the absorbance of the oxidized o-dianisidine in Blank sample,

ΔAC = change in the absorbance of the oxidized o-dianisidine in Control sample,

C = concentration of oxidized o-dianisidine in Compost sample, moles L^{-1},

CC = concentration of oxidized o-dianisidine in Control sample, moles L^{-1},

CB = concentration of oxidized o-dianisidine in Blank sample, moles L^{-1},

CN = concentration of oxidized o-dianisidine in compost as a result of catalysis by peroxidase, moles L^{-1},

25 = volume of sample, mL,

1.1296 = dilution of sample in cuvette,

$1{\times}10^6$ = conversion from moles to μmoles

D = μ*M* of oxidized o-dianisidine in compost mL^{-1}.

H = activity of peroxidase, μM of oxidized o-dianisidine in compost mL^{-1} 30 s^{-1}

M = ratio of oven dried sample weight versus as-received weight, unitless.

05.04 SUMMARY OF METHODS

32. Report

32.1 *Method 05.04-A Phosphatases*:

32.1.1 Report activity of phosphatases in μg of *p*-Nitrophenol g^{-1} of oven dried compost h^{-1} method used for determination.

32.1.2 *Additional Information*—Report compost pH, total solids, type of source material (e.g., municipal solid waste, yard waste, biosolids, etc.), and maturity rating.

32.2 *Method 05.04-B Dehydrogenases:*

32.2.1 Report activity of dehyrogenase in mg of triphenyl formazan g^{-1} of oven dried compost 24 h^{-1}.

32.2.2 *Additional Information*—Report compost pH, total solids, type of source material (e.g., municipal solid waste, yard waste, biosolids, etc.), and maturity rating.

32.3 *Method 05.04-C Proteases:*

32.3.1 Report activity of protease in μmoles of leucine g^{-1} of oven dried compost h^{-1}.

32.3.2 *Additional Information*—Report compost pH, total solids, type of source material (e.g., municipal solid waste, yard waste, biosolids, etc.), and maturity rating.

32.4 *Method 05.04-D Cellulases:*

32.4.1 Report activity of cellulase in mg of glucose g^{-1} of oven dried compost 24 h^{-1}.

32.4.2 *Additional Information*—Report compost pH, total solids, type of source material (e.g., municipal solid waste, yard waste, biosolids, etc.), and maturity rating.

32.5 *Method 05.04-E Peroxidases:*

32.5.1 Report activity of peroxidases in μmoles of hydrogenperoxidase (H_2O_2) g^{-1} of oven dried compost 30 s^{-1}.

32.5.2 *Additional Information*—Report compost pH, total solids, type of source material (e.g., municipal solid waste, yard waste, biosolids, etc.), and maturity rating.

33. Precision and Bias

33.1 The precision and bias of these tests have not been determined. Data are being sought for use in developing a precision and bias statement.

34. Keywords

34.1 N-acetyl-ß-glucosaminidase; arylsulphatase; L-asparaginase; amidase; cellulase; dehydrogenase; enzyme; enzyme activity; enzymes; ß-glucosidase; ß-galactosidase; laccase; lignin peroxidase; manganese peroxidase; phosphatase; Phosphoric monoester hydrolase; phosphodiesterase; protease

Test Method: Biological Assays. Three Methods							**Units:** method specific	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								05.05-A
								05.05-B
								05.05-C

05.05 BIOLOGICAL ASSAYS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers qualitative bioassay techniques to screen for the presence of phytotoxins in compost using seed germination and growth.

1.1.1 *Method 05.05-A Seedling Emergence and Relative Growth.*

1.1.2 *Method 05.05-B In-Vitro Germination and Root Elongation.*

1.1.3 *Method 05.05-C Earthworm Bioassay: Minnesota "Z" Test.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC Methods:*

Method 03.10-E Quick-Test to Approximate Water-Holding Capacity of Compost

Method 03.09-A Total Solids and Moisture at 70±5°C.

2.2 *Other Sources:*

Iannotti, D. A., M. E. Grebus, B. L. Toth, L. V. Madden, and H. A. J. Hoitink. 1994. Oxygen Respirometry to Assess Stability and Maturity of Composted Municipal Solid Waste. J. Environ. Qual. 23:1177-1183.

OECD Guideline for Testing of Chemicals 208. 1984. Terrestrial Plants, Growth Test. *Update:* Terrestrial (Non-Target) Plant Test. 208 A: Seedling Emergence and Seedling Growth Test, and 208 B: Vegetative Vigour Test.

US EPA 744-R-00-010. August 2000. Sampling and analysis of consumer garden products that contain vermiculite.

Zachmann, J., and T. R. Halbach. 1991. Testing compost for toxicity and stability using an earthworm bioassay: The Minnesota "Z" TEST. University of Minnesota, St. Paul, MN 55108.

3. Terminology

3.1 *bioassay, n*—A screening test to determine the relative effects of a product by comparing its impact on a test organism with that of a standard preparation or control.

3.2 *cotyledon, n*—A leaf of the embryo of a seed plant, which, upon germination either remains in the seed or emerges, enlarges, and becomes green. Also called seed leaf.

3.3 *dilution extract, n*—The solution that remains after separation of compost from water contains various salts and soluble organic compounds. This extract is further diluted by mixing a measured volume into a measured volume of distilled water, (e.g., 1 mL of extract mixed with 9 mL of water is a 1:10 dilution extract).

3.4 *etiolation, n*—Excessive elongation of the hypocotyl of a plant after emergence because of limited exposure to sunlight.

3.5 *germination, n*—Begin to sprout or grow, as with a seed.

3.6 *hypocotyl, n*—The part of the axis of a plant embryo or seedling plant that is below the cotyledons.

3.7 *phytotoxicity, n*—Poisonous to plants, chemical damage to a growing plant or emerging seedling.

3.8 *radicle, n*—The part of a plant embryo that develops into a root.

3.9 *root, n*—The usually underground portion of a plant that serves as the conduit through which nutrients,

minerals and water from the surrounding soil are taken up and used for growth and metabolism; it also anchors the plant and may store nutrients, (e.g. potatoes).

4. Summary of Test Methods

4.1 *Method 05.05-A Seedling Emergence and Relative Growth*—Seedling emergence and relative vigor in potting media through direct seeding using distilled water as the moisture source. Cucumber seeds are planted in a blend of 50% compost and 50% No. 2 grade vermiculite. Two controls are recommended: pure vermiculite; and a soilless potting media. The ratio of emerged seedlings to the number of seeds planted indicates percent emergence. Relative seedling health and vigor is evaluated by use of the following indicators: seedling turgor, hypocotyl length (stunting) and relative distortion or shape of cotyledons, and fresh weight of shoots.

4.1.1 Cucumber is a good indicator species for this test because of its high salt tolerance, large seed size, distinct cotyledon shape, and intolerance to volatile fatty acids.

4.2 *Method 05.05-B In-Vitro Germination and Root Elongation*—This method covers two germination parameters: germination rate and root (radicle) elongation.

4.2.1 Germination rates of cucumber seed subjected to a compost extract solution are compared to the germination rate of cucumber seed in deionized water.

4.2.2 Radicle elongation of germinated cucumber seeds subjected to a compost extract solution is compared to radicle elongation of cucumber seed in deionized water.

4.3 *Method 05.05-C Earthworm Bioassay: Minnesota "Z" TEST*—The bioassay consists of placing *Eisenia fetida* (earthworms) in samples of compost with and without added cellulose. The mass of *E. fetida* is recorded before and after two seven-day exposure periods. The test compost is considered toxic if earthworms die after exposure. The test compost is considered unstable if earthworm weight gain of more than 40% is observed in both treatments.

5. Significance and Use

5.1 Germination tests are simple, straightforward tests that screen for the presence of phytotoxic materials and substances. Seed germination tests can indicate the presence of significant quantities of plant toxins that are sometimes found in composts.

5.2 *Method 05.05-C Earthworm Bioassay: Minnesota "Z" TEST—Eisenia fetida* is an inexpensive and rapid bioindicator of compost stability and toxicity. The earthworm is an ideal test species because it commonly inhabits and ingests composting organic material during the middle and late stages of the composting process.

6. Interference and Limitations

6.1 Biological assays are not stand-alone indicators of compost maturity, so other indicators must be considered such as C:N ratio, respirometry, ammonium to nitrate ratio, etc.

6.1.1 Biological assays generally do not differentiate among growth inhibition causes, (e.g., by phytotoxicity from ammonia, salts, organic acids, high or low pH, etc.), or apparent soil nutrient deficiency such as N caused by high C:N composts. Additional testing is needed to isolate and identify specific causes of phytotoxicity.

6.2 Seedling development is retarded when grown in the presence of toxic compounds that are common to immature composts. Seedling mortality or stunting may occur under phytotoxic conditions. Ammonia, soluble salts, and organic acids at high concentrations will inhibit root development and/or nutrient uptake.

6.2.1 Some species including many within the cucurbit family are relatively salt tolerant and are not prone to serious salt damage, but are susceptible to various volatile organic compounds found in some composts.

6.2.2 Other species, (e.g., cress), are salt intolerant, prone to serious salt damage, and are susceptible to various volatile organic compounds found in immature or raw composts.

6.2.3 Small seeds and their seedlings, such as timothy, are time consuming to count and difficult to see.

6.3 *Method 05.05-A Seedling Emergence and Relative Growth*—Compost is blended with vermiculite as if it were a peat substitute in a commercial potting mix used for seedling market flat production.

6.3.1 Reproducibility of results are low when strict guidelines for soilless potting media and positive control replicate preparation are not followed.

6.3.2 Vermiculite must be segregated by sieve size to create a homogeneous amendment, (e.g., large particles will create excessively large pore spaces that may cause moisture stress and retard seedling development).

6.3.2.1 Vermiculite[1] is a phyllosilicate mineral widely used as a component of horticultural potting mix for plant growth. It is stable and contributes K, Mg, and a number of minor elements. It is useful as a carrer and

[1] The US EPA recommends moistening vermiculite to minimize exposure to potentially asbestos-contaminated vermiculite dust. *Source:* US EPA 744-R-00-010. August 2000.

extender for fertilizers and other agochemicals because of its high absorptive capacity. In the compost bioassay in which vermiculite is used as the compost diluent, the absorptive capacity of the vermiculite could reduce the phytotoxin content of the bioassay mix, but any practically significant remaining phytotoxins would be observable in the growth response of the test plants. The bioassay test is not intended to measure the presence of phytotoxins in absolute terms, rather it is designed to provide the user with an assessment of obvious negative growth response from an excessively immature material with large amounts of phytotoxins and with compost in a simulated likely usage situation, such as a horticultural mix with vermiculite.

6.3.2.2 Some vermiculites are very high in soluble, and potentially phytotoxic compounds. It is therefore necessary to thoroughly rinse the suspected vermiculite with deionized water to remove the soluble compounds. The negative control is recommended to screen for the presence of problem vermiculites.

6.4 *Method 05.05-B In-Vitro Germination and Root Elongation*—Compost extracts will contain salts and possibly other phytotoxins if compost is immature.

6.5 *Method 05.05-C Earthworm Bioassay: Minnesota "Z" TEST*—This bioassay is a screening test that indicates the presence of significant quantities of toxins that are sometimes found in composts. It does not determine the sources or causes of toxicity. Additional testing is required to isolate and identify specific toxicants.

7. Sample Handling

7.1 Prepare as-received moist compost for these tests. Screening will improve precision, but can diminish accuracy in extreme cases.

Test Method: Biological Assays. Seedling Emergence and Relative Growth						**Units:** % of control		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
								05.05-A

05.05-A SEEDLING EMERGENCE AND RELATIVE GROWTH

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

PRECAUTION—The US EPA recommends moistening vermiculite to minimize exposure to potentially asbestos-contaminated vermiculite dust. *Source:* US EPA 744-R-00-010. August 2000.

8. Apparatus for Method A

8.1 *Plastic Seedling flat*—flats with 162 cells (9 × 18 cells).

8.2 *Plant Grow Lights*—fixture fitted with grow-light bulbs connected to 24-h timer.

8.3 *Plastic Bags*—approximately 50-L, clear polyethylene, (e.g., GLAD® QUICK-TIE®, Clear Recycling Tall Kitchen Bags, 13-gal – email: glad@firstbrands.com for distributors and their locations).

9. Reagents and Materials for Method A

9.1 *Water*—distilled.

9.2 *Vermiculite*—No. 2 grade, thoroughly rinsed with DI water.

9.3 *Potting Media*—commercial, available in garden stores, (e.g., MetroMix - W.R.Grace, or equal quality).

CAUTION !—Do not use soil. Use a proven soilless potting mix with peat moss.

9.4 *Cucumber Seeds*—Select a commonly available, salt tolerant variety, (e.g., Marketmore 76 variety. Jordan Seeds, Inc.; 6400 Upper Afton Road; Woodbury, MN 55125).

10. Procedures for Method A

10.1 *Media Preparation and Seeding:*

10.1.1 Completely saturate a 300 cm^3 aliquot of vermiculite with deionized water. Allow the vermiculite to absorb as much water as possible, allow at least four hours. Gravity-drain all excess water; properly moistened vermiculite will feel wet, but not produce free water.

10.1.2 Transfer a 300 cm^3 aliquot of as-received compost into a 4-L (1-gal) mixing container, (e.g., a plastic bag).

10.1.2.1 *Squeeze Test*—A squeeze test is performed with a handful of compost. A moist sample will clump when tightly squeezed. A sample with optimal moisture will feel wet, but not produce free water. A sample that is too dry is dusty and will not clump with hard squeezing.

10.1.2.2 Moisten the compost aliquot as necessary to optimize compost moisture, i.e., to feel wet, but not produce free water.

10.1.3 Blend equal volumes of pre-moistened vermiculite and test material. Mix thoroughly by rotating and shaking the bag,

10.1.4 *Prepare the seedling flats*—Fill three adjacent 9-cell rows of the seedling flat with the blended compost-vermiculite mixture representing each sample [3 × 9 cells], Fig 05.05-A1.

CAUTION—The media can fall through the drainage hole that pierces the bottom of each seedling flat cell. Appropriate measures should be taken to minimize media loss through the holes.

10.1.4.1 *Positive Control*—Fill two randomly assigned 9-cell rows of the seedling flat with the pure soilless potting media [2 × 9 cells]. Each row of pure potting media is a *positive control* replicate. Do not position both replicates adjacent to the same compost sample, i.e., as neighbors of the same compost sample.

10.1.4.2 *Negative Control*—Fill one randomly assigned 9-cell rows of the seedling flat with the pure vermiculite [1× 9 cells]. Each row of pure vermiculite is a *negative control* replicate.

10.1.4.3 *Control Replicates*—Always include the positive and negative controls in each seedling flat.

10.1.5 Place two cucumber seeds in each cell, covering the seeds with approximately 1 cm of material.

10.2 *Control Growing Conditions:*

10.2.1 Place the seedling flat in a clear or translucent plastic bag.

10.2.2 Fill the bag with air and seal to prevent air loss or leakage and to conserve moisture throughout the duration of the experiment.

NOTE 1A—If plastic bags fail by leaking air and flattening, construct a skeletal structure for the bag from wire or other rigid material and place the support structure inside of the bag for the duration of the experiment.

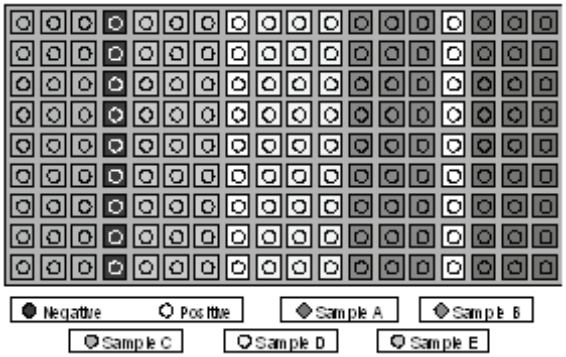

Fig 05.05-A1 Diagram of a 162-cell seedling flat [9 × 18 cells]. One seedling flat will accommodate up to five separate compost samples, plus one negative control and two positive controls.

10.2.3 Place each seedling tray under a florescent light fixture with the 24-h timer. Set the timer to 14 h of continuous light and 10 h of continuous darkness. Make sure that the entire tray is illuminated uniformly.

10.2.4 Allow approximately 12 to 14 d for growth, periodically checking plastic bags for adequate inflation and proper moisture.

10.2.5 Maintain constant room temperature of approximately 27°C.

10.3 *Evaluation of Emergence:*

10.3.1 After 12 to 14 d or after the first true leaf begins to unfold, remove the seedling flats from plastic bags.

10.3.2 Count and record the number of seedlings within each row that have a fully exposed hypocotyl and fully expanded cotyledons.

10.4 *Evaluation of Relative Growth and Vigor*

10.4.1 *Positive Control*—Carefully study the morphology of all seedlings in the two positive control rows. Measure the height of positive control seedlings and determine their average height. Take special note of the cotyledon turgidity and cuticles smoothness.

CAUTION !—If one of the two replicates of the positive control appears stunted or has distorted cotyledons, disregard that row and use only the "healthy" seedlings as the health and vigor reference standard.

10.4.2 *Seedling Health and Vigor:*

10.4.2.1 The height of a *healthy, vigorous* seedling is the same or greater than the average seedling height of the positive control.

10.4.2.2 A healthy vigorous seedling is turgid; it has a fully extended hypocotyl and its cotyledons are smooth, not deformed or wrinkled.

10.4.3 Count and record the number of *healthy, vigorous* seedlings within each row.

11. Trouble Shooting for Method A

11.1 Avoid pooling of water around the base of the seedling flats inside of the closed bag; excessive moisture can promote the development of phytotoxic, anaerobic conditions.

11.2 The *negative control* is included to verify that the vermiculite contains insignificant concentrations of phytotoxic compounds. If severely limited hypocotyl elongation and severe distortion of cotyledons is observed, use deionized water to thoroughly rinse and then drain and dry the vermiculite before blending it with the compost samples.

11.3 Provide uniform lighting/shading to minimize non-uniform etiolation.

11.4 Addition of excess water during the media preparation steps can diminish the impact negative growth factors and affect false positives.

12. Calculations for Method A

12.1 *Determine Emergence, %:*

$$E = ES \div C \times 100 \qquad \textit{Equation 12.1}$$

where:

E = relative percent emergence, % of control,
ES = number of cucumber seedlings with exposed cotyledons and hypocotyl, and
C = number of emerged control seedlings.

12.2 *Determine Relative Seedling Vigor, %:*

$$V = VS \div C \times 100 \qquad \textit{Equation 12.2}$$

where:

V = relative percentage of healthy, vigorous, % of control,
C = number of healthy, vigorous control seedlings, and
VS = number of cucumber seedlings with well formed, un-deformed cotyledons, and turgid hypocotyl with a length equal to or greater than the positive control average.

13. Interpretation of Results for Method A

13.1 This bioassay is a test to screen for the presence of a phyotoxic condition and should not be used as the sole indicator for determining the end use of a compost product. Additional tests to complement the bioassay outcome should be performed on parallel sample aliquots, (e.g., electrical conductivity, pH, NH_4-N plus NH_3-N, inorganic carbon or carbonates, respirometry, total nitrogen, sulfates and sulfites, etc.).

Table 05.05-A1. Maturity Indicator Rating.

Test Parameters	*Very Mature*	*Mature*	*Immature*
Emergence, %[1]	> 90	90 – 80	< 80
Seedling Vigor, %	> 95	85 – 95	< 85

[1] %, percentage relative to positive control (step 10.4). Never base end-use conclusions on the result of a single test.

13.2 End use instructions for a compost must be based upon application technique and the analytical results for a full suite of test parameters.

13.3 Verify bioassay outcome using additional testing. Cucumber seedlings grown in a compost with a relatively high electrical conductivity reading (e.g., > 8 dS m^{-1}) will be stunted (diminished etiolation), have a deeper green color and a thicker cotyledon cutical than the positive control seedlings. Presence of these symptoms must be verified with an electrical conductivity test using a parallel aliquot of the test sample in question.

NOTE 2A—Electrical conductivity readings are often exagerated in samples where carbonates and/or ammonium-plus ammonia-nitrogen concentrations are high.

13.4 Alternative plant species for use as bioassays are outlined in OECD Guideline for Testing of Chemicals 208 (1984). A representative plant species should be selected for use with specific compost uses, (e.g., greenhouse potting mixes, land applications, etc.).

Test Method: Biological Assays. In-Vitro Germination and Root Elongation						**Units:** % of control		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
								05.05-B

05.05-B IN-VITRO GERMINATION AND ROOT ELONGATION

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTED BY—Dr. Aziz Shiralipour, University of Florida, Gainsville, FL.

14. Apparatus for Method B

14.1 *Petri Dishes*—9 cm diameter, glass or plastic.

14.2 *Germination Paper*—9 cm diameter disks to fit inside Petri dish, Whatman No. 1 or No. 3.

14.3 *Stirring Rod*—glass, metal or plastic.

14.4 *Bottle with Cap*—100-mL, plastic or other.

14.5 *Evaporation Dish*—150-mL oven-proof beaker.

14.6 *Drying Oven*—forced air, vented, 70±5°C.

14.7 *Analytical Balance*—capable of weighing 150 g, accurate to ±0.001 g.

14.8 *Desiccator*—equipped with calcium chloride as a desiccant, (e.g., Fisher Scientific, or equal).

15. Reagents and Materials for Method B

15.1 *Water*—distilled.

15.2 *Cucumber Seeds*—Select a commonly available, salt tolerant variety, (e.g., Marketmore 76. Jordan Seeds, Inc.; 6400 Upper Afton Road; Woodbury, MN 55125).

16. Procedures for Method B

16.1 *Determine Total Solids:*

16.1.1 Transfer 50 cm^3 of as-received compost to tared 150-mL beaker.

16.1.2 Weigh and record as-received net weight, ±0.001 g.

16.1.3 Oven-dry sample at 70±5°C for 18 to 24 h, until sample weight stabilizes and evaporative water loss diminishes to nil.

16.1.4 Weigh and record net oven-dry weight of sample, ±0.001 g.

16.1.5 Calculate total solids ratio for use in calculating water to compost ratio during extract preparation.

16.2 *Prepare Extract:*

16.2.1 Add two parts distilled water to one part compost (dw basis) in clean 150-mL beaker.

16.2.1.1 Mix well and allow compost to soak for 3 h.

16.2.1.2 Stir well 2× to 3× during soaking period.

16.2.1.3 Release extract by compressing compost in the beaker with stirring rod.

16.2.2 Filter mixture through filter paper.

16.2.3 Collect extract in clean bottle or container for phytotoxicity test.

16.3 *Prepare Petri Dishes:*

16.3.1 Place 10 cucumber seeds onto germination paper or filter paper in a 9-cm petri dish.

16.3.2 Add 10 mL of compost extract, step 16.2.3.

NOTE 1C—Germination paper should be saturated with compost extract, with a thin layer of extract at its surface.

NOTE 2C—Size of petri dish may vary, in which case amount of compost extract should vary proportionately.

16.4 *Standard Reference*—As a control, substitute distilled water for compost extract in 3 to 5 petri dishes.

16.5 *Sample Treatments:*

16.5.1 Place petri dishes in lighted area, but not in direct sunlight to avoid rapid evaporation.

16.5.2 Compare cucumber seed germination time and root length in assays conducted with compost extract to those conducted with distilled water, at 5 and 7 d.

17. Calculations for Method B

17.1 *Relative Germination Rate:*

$$G = A \div B \times 100 \qquad \textit{Equation 17.1}$$

where:

G = germination rate of treated seed relative to control, % d d^{-1},
A = average germination time for treated seeds, d, and
B = average germination time for seeds in distilled water (control), d.

17.2 *Relative Root Elongation*:

$$E = C \div D \times 100 \qquad \textit{Equation 17.2}$$

where:

E = relative root length of treated seed relative to control, % mm mm^{-1},
C = average root length of treated seeds, mm, and
D = average root length of control seeds, mm.

Test Method: Biological Assays. Earthworm Bioassay: The Minnesota "Z"-Test							**Units:** %, Δ g g^{-1}	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
								05.05-C

05.05-C EARTHWORM BIOASSAY: THE MINNESOTA "Z"-TEST

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

SUBMITTED BY—Thomas R. Halbach; Department of Soil, Water, and Climate; University of Minnesota, St. Paul.

18. Apparatus for Method C

18.1 *Analytical Balance*—capable of measuring mass of up to 100 g, ±0.001 g for use in measuring mass of test-worms.

18.2 *Flourescent Lighting*

18.3 *Funnels*—two glass, 10-cm diameter mouth and 7.5-cm neck.

18.4 *Scale*—capable of measuring mass of up to one kg, ±1.0 g for use in preparing sample mixes.

18.5 *Test Container*—1-L capacity, (e.g., Ziploc® storage container).

19. Reagents and Materials for Method C

19.1 *Cellulose*—100% cellulose powder, (e.g., available through Fischer Scientific, etc.).

19.2 *Eisenia fetida*—six earthworms, each weighing approximately 100 mg to 200 mg.

19.3 *Filter Paper*—cellulose, acid- and nitrogen-free, 20-cm diameter to fit glass funnels.

19.4 *Sand*—silica sand, sieve size 0.5-mm to 2.0-mm, contaminant-free, rinsed with deionized water and air-dried.

19.5 *Water*—deionized, minimum resistivity, 17 MΩ·cm, minimum standard.

20. Procedures for Method C

20.1 *Sample Preparation:*

20.1.1 Determine sample water-holding capacity and total solids content on a parallel aliquot of the compost test sample. Refer to Method 03.10-E Quick-Test to Approximate Water-Holding Capacity of Compost; and Method 03.09-A Total Solids and Moisture at 70±5°C.

20.1.2 Moisten two 25-g dry weight equivalent aliquots of the test compost to approximately 120% water-holding capacity (dw basis).

20.1.2.1 Determine the dry-weight equivalent aliquot size as follows:

$$A = B \div [C \times 0.01] \qquad \textit{Equation 20.1.2.1}$$

where:

A = mass of as-received moist compost aliquot, g
B = required dry-weight equivalent aliquot size, 25.0 g,
C = sample total solids content, % wet weight basis, and
0.01 = factor to convert from percentage to fraction, unitless.

20.1.3 Add and mix 125 g of washed, air-dried sand into each of the two moistened compost aliquots.

20.1.3.1 *Positive Control*—Add 10 g of cellulose power plus 30 mL of additional water to one compost test aliquot and label to indicate positive control containing cellulose.

20.1.3.2 *Test Sample*—Do not include cellulose powder nor additional water to the second test aliquot of compost. Label to indicate test sample.

20.2 *Earthworm Preparation*

20.2.1 *Initial Earthworm Mass*—Weigh and record the mass of each of six earthworms. Each earthworm must weigh between 100 and 200 mg.

20.2.2 Transfer three earthworms to each of the two test aliquots. Record the combined mass of earthworms of each test aliquot.

20.2.3 *Incubation*—Place the prepared sample containers under constant fluorescent lighting and maintain at ambient laboratory temperature, approximately 23°C.

20.3 *Measurements and Observations:*

20.3.1 *7-Day Earthworm Mass*—Remove the earthworms after seven days. Weigh and record their combined weight.

20.3.1.1 *7-Day Mortality*—Count and record the number of live earthworms retrieved and weighed.

20.3.2 Return the earthworms to the test container and resume incubation as described.

20.3.3 *14-Day Earthworm Mass*—Remove the earthworms after seven more days. Weigh and record their combined weight.

20.3.3.1 *14-Day Mortality*—Count and record the number of live worms retrieved and weighed.

21. Calculations for Method C

21.1 Calculate the percent change in the combined earthworm mass for each test period (7 d) and for each of the two test aliquots, i.e., the positive control and test sample:

$$G_i = [F_j - I_i] \div I_i \times 100 \qquad \textit{Equation 21.1}$$

where:

G_i = percent gain (positive) or loss (negative) in earthworm weight for each of the two test sample aliquots (i), i.e., the positive control containing additional cellulose and the test sample, %, g g^{-1},

F_j = combined mass of surviving earthworms at the end of each test period (j), steps 20.3.1 and 20.3.3, g, and

I_i = combined mass as measured at earthworm preparation for each of the two test aliquots (i), step 20.2.2, g.

22. Interpretation of Results for Method C

22.1 Compost is considered unstable if earthworm weight change is greater than forty percent for both positive control (cellulose added) and the test sample with no added cellulose for either the 7-day or 14-day exposure period.

22.2 Compost is considered stable if earthworm weight change is greater than forty percent in the positive control (cellulose added) and earthworm weight change is between zero and forty percent in the test sample with no added cellulose.

22.3 Compost is considered toxic when earthworm weight change of either sample is negative, less than zero percent, or when earthworm mortality is observed for either treatment.

22.4 *Replicate earthworm mortality:*

22.4.1 Run the bioassay again to verify the results;

22.4.2 Run an alternative bioassay parallel to the earthworm bioassay and compare the results; or

22.4.3 Submit a subsample of the same compost to a laboratory for chemical analysis to test for potentially toxic conditions or suspected compounds, (e.g., pH, NH_4, heavy metals, organic contaminants, etc.).

05.05 METHODS SUMMARY

23. Report

23.1 *Method 05.05-A Seedling Emergence and Relative Growth*—Report percentage of emerged seedlings and percentage of vigorous seedlings. Report percent emergence for both positive and negative controls.

23.1.1 Percent emergence indicates the number of emerged seedlings relative to the number of seeds planted for a treatment.

23.1.2 The percent of vigorous seedlings indicates the number of turgid seedlings with well formed cotyledons present, relative to the number of seeds planted.

23.2 *Method 05.05-B In-Vitro Germination and Root Elongation*—Report delayed germination (d) and reduction in root length (cm) relative to deionized controls.

23.2.1 Any delay in seed germination and reduction in root length will be due to presence of phytotoxic material.

23.2.2 If no phytotoxic material is present, the compost extract-treated seeds should germinate at the same time as the seeds germinate in pure distilled water. The size of the roots should also be the same.

23.3 *Method 05.05-C Earthworm Bioassay: The Minnesota "Z"-Test*—Report the percent change in earthworm weight for each of the two test aliquots for the 7-day and 14 day test periods. Report earthworm mortality when observed.

24. Precision and Bias

24.1 The precision and bias of the following tests have not been determined. Data are being sought for use in developing a precision and bias statement.

24.1.1 *Method 05.05-A Seedling Emergence and Relative Growth*

24.1.2 *Method 05.05-B In-Vitro Germination and Root Elongation*.

24.1.3 *Method 05.05-C Earthworm Bioassay: The Minnesota "Z"-Test*.

25. Keywords

25.1 germination; root elongation; turgor; cotyledon; hypocotyl; emergence; seedling development; earthworms, "Z" test

Test Method: Odor. Two Methods						**Units:** unitless		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.06-A		05.06-A		05.06-A		
			05.06-B					

05.06 ODOR

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section reviews test protocols for making qualitative and semi-quantitative measures of compost odor emissions.

1.1.1 *Method 05.06-A Field Assessment of Compost Odor.*

1.1.2 *Method 05.06-B Field Sampling of Biofilter Odor Emissions*.

1.1.3 *Appendices:*

1.1.3.1 *Appendix I 05.06 Example of Performance Standards for Odorous Emissions from a Permanent Constructed Facility.*

1.1.3.2 *Appendix II 05.06 Odor notification Form.*

1.1.3.3 *Appendix III 05.06 Resident Odor Complaint Form.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 05.03-A *Quick-Test* for Field Assessment of Compost Color and Odor.

2.2 *Other References:*

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3. Terminology

3.1 *ascending scale of concentrations, n*—A series of increasing concentrations of an odorous substance in a chosen medium.

3.2 *blank sample, n*—A quantity of the medium that is used to dilute samples.

3.3 *detection threshold (DT), n*—The lowest concentration of a substance in a medium relating to the lowest physical intensity at which a stimulus is *detected* as determined by the dilutions-to-threshold best-estimate criterion.

3.4 *dilutions to threshold (D/T), n*—The volume ratio of carbon-filtered or clean ambient air to odorous air (odor) at which the individual detects an odor.

3.5 *maturity, n*—The degree to which a compost product is free of phytotoxic substances that can cause delayed seed generation, plant damage, or seed and plant death. Maturity is measured through plant bioassays, such as seed germination, root elongation and plant growth trials. Negative plant effects due to immaturity are caused by a build-up of short chain volatile fatty acids that are odorous, i.e. acetic, proprionic, isobutyric and butyric, isovaleric and valeric. Most plant bioassays can also identify high levels of other phytotoxic substances. When composts are properly produced and stored, the build-up of these substances is not significant.

3.6 *odor threshold, n*—The minimum concentration required for an individual to perceive a specific odorant.

3.6.1 *threshold odor concentration, TOC, n*—The minimum concentration of odorant that elicits the perception of odor; a term from odor of water testing methods (SM 2150).

3.7 *odor, n*—The property or quality of a thing that affects, stimulates, or is perceived by the sense of smell.

3.8 *odorant, n*—A volatile compound that affects, stimulates, or is perceived by the sense of smell, a compound that generates odor. Compounds most commonly defined as odorants are described below:

3.8.1 *amines*—These are compounds that contain amino groups ($-NH_2$), (e.g., methylamine, ethylamine, trimethylamine, and diethylamine). They can be produced in easily detectable quantities during high temperature processes. Amines can be produced along with ammonia, and if chlorine is present, chloramines may be released. In composting, amines result from microbial decomposition that involves decarboxylation of amino acids. The amines that are produced are easily volatilized when temperatures are elevated above about 27°C. High ambient temperatures can accentuate volatilization of amines that may be microbially split off from the core backbone of polymers used to flocculate biosolids.

3.8.2 *ammonia*—Ammonia is most often found in emissions from fresh or early phase composting materials that are pH 8.0 or greater. Ammonia emission comes from anaerobic bacterial digestion of proteins in the materials. Ammonia release increases as pH increases above 8.0. Ammonia is often

accompanied by release of amines, and if chlorine is used, chloramines may be released as well.

3.8.2.1 Ammonia also has an important special characteristic that facility operators need to recognize. At high concentrations, it is so intense that it strongly masks odors from other compounds, even those containing reduced sulfur groups. At least 100 to 1000 times more ammonia than reduced sulfur compound is needed per unit volume of air for an average person to detect it. Such high concentrations can occur at some facilities depending on the feedstocks and operations.

3.8.2.2 On-site assessments for potential off-site odors could be misleading if on-site detection were limited to ammonia. Reduced sulfur compounds also might be present, but undetectable because of ammonia masking. As the air 'parcel' containing both types of compounds moves downwind, beyond the facility site perimeter, ammonia would be diluted below its detection threshold. In contrast, the reduced sulfur compounds, although also diluted below their on-site concentrations, may still be concentrated enough to remain above their detection thresholds. For this reason, odor assessments at facilities might also benefit from some monitoring for off-site odors typical of reduced sulfur or amine compounds.

3.8.3 *inorganic sulfur*—Compounds such as hydrogen sulfide. The latter (H_2S) is often the focus of discussion because of its association with the familiar rotten egg odor. However, it is rarely detected in stockpiles or well-managed composting operations. Often other compounds or combinations of compounds are the primary cause of odor. Increasing the pH to 9.0 or higher, as with lime stabilized biosolids, can eliminate H_2S emissions.

3.8.4 *organic sulfur*—compounds such as dimethyl disulfide (DMDS) and dimethyl sulfide have been associated with odorous emissions from biosolids composting operations. Also, they have been measured at wastewater dewatering, solids, pelletizing, and digestion facilities. In general, compounds like DMDS are by-products of chemical or microbial degradation (anaerobic) of proteins.

3.8.5 *mercaptans or thiols*—are a generic class of straight-chained organic compounds containing a single sulfur molecule. Methyl mercaptan is the most common thiol released from biosolids; it has a low odor detection threshold, i.e., small amounts are easily detectable. Two methyl mercaptan molecules combine to form one dimethyl disulfide (DMDS) molecule. Garlic (allyl sulfide), onions (propanethiol), and spoiled broccoli contain mercaptan-like compounds. The boiling point of methyl mercaptan is 6°C, which makes it a gas at room temperature. Therefore, collection techniques that use tedlar bags are acceptable.

3.8.6 *volatile fatty acids, VFAs*—These short chain (<C8) fatty acids have the general formula $C_nH_{2n+1}COOH$ and are typically generated during anaerobic decomposition of vegetable materials, such as hay, straw, grass, leaves, silage, etc. They also volatilize from livestock manure. VFAs include formic, acetic, propionic and lactic, butyric and iso-butyric, valeric and iso-valeric, caproic and iso-caproic, and heptanoic acids. Refer to Method 05.10 Volatile Fatty Acids for more complete chemical descriptions and detection techniques for these compounds. VFAs are volatile and are subject to rapid microbial decomposition under aerobic conditions. Production of phytotoxic quantities of VFAs during composting, prior to compost maturation, is known to occur. The VFAs are most likely to be involved in odorous emissions when vegetative matter is present, such as occurs in the first stages of a composting operation when grass and green matter are delivered and sorted. They are unlikely to occur with biosolids alone.

3.9 *panelists, n*—Individuals whose odor thresholds are being evaluated, or who are utilized to determine the odor threshold of the substance of interest.

3.10 *recognition threshold, n*—The lowest concentration of a substance in a medium relating to the lowest physical intensity at which a stimulus is *recognized* as determined by the best-estimate criterion.

3.11 *sample, n*—A material in any form that may or may not exhibit an odor, depending on the amount of odorous components that it may contain.

3.12 *scale steps, n*—Discrete concentration levels of a substance in a medium, with concentrations increased by the same factor per step throughout the scale.

3.13 *smell, v*—To perceive the scent of (something) by means of the olfactory nerves.

3.14 *stability, n*—A general term that refers to the biological activity state of compost and associated available carbon content; typically greatest stability occurs after the final stage in the composting process. Desirable high stability compost is characterized by a relatively lower and constant rate of microbial respiration than in previous stages and by a corresponding decrease of VOCs. As a result, stabilized compost does not generate malodorous compounds.

3.15 *standard sample, n*—The medium to which an odorous material has been added at a known concentration.

4. Odor Determination

4.1 *Odor Sample Collection*—Odor sample collection for subsequent chemical or olfactometry analysis is used for routine monitoring of compliance with an air pollution regulatory limit, and at a facility that has been unable to resolve odor emissions problems by other means and needs specific odor constituent data. In such a case, facility operators may pursue analytical strategies upon which to base a remediation program. The proper collection of an air sample containing odorous compounds is essential for accurate analysis of the intensity and source of the odor. This is true for both qualitative and quantitative methods of odor analysis. Compost facilities can be expected to generate a complex mixture of compounds only, some of which may have a potential for creating a problem if process management is inadequate. The components of the odor will often dictate the method of sampling. Therefore, insight as to which compounds or type of compounds may be contributing to the odor is desirable. Without this, a sampling method that can handle a broad range of compounds would be necessary. After identifying the type or group of odorants present, an appropriate sampling method can be used.

4.2 Physical and chemical properties of the odorant, e.g., polarity, volatility, and stability, will often determine which sampling method is desirable Condensation, adsorption, or permeation of the odorous compounds through the walls of the collection system can cause errors.

4.2.1 For example, DMDS is a liquid at ambient temperature, c.f., the boiling point of DMDS is 109°C. This physical property greatly influences DMDS emissions and measurement. Elevated temperatures will dramatically increase DMDS emissions that are odorous even at very low concentrations below saturation.

4.2.2 DMDS and other compounds with a high vapor pressure will not condense if the vapor is present below the saturation concentration. It is important to use sampling techniques that do not allow the sample to cool before it enters the analytical detector as a precaution to prevent water vapor from condensing onto the walls of the sample bag.

4.2.2.1 DMDS will not condense if the vapor is present below its saturation concentration. In almost all cases, because it takes so little DMDS to be odorous, the concentration will be less than saturation.

4.3 Gas canisters, Tedlar® bags, flux chambers, and adsorbent tubes are used to collect odor samples. Adsorption tubes filled with Tenax® packing and/or activated carbon are common types of traps used for ambient air sampling. Industrial hygienists often use adsorbent tubes for on-site analysis of specific individual compounds like ammonia, hydrogen sulfide, etc.

5. Sample Analysis

5.1 The ability to detect, identify, and quantify odorants in compost is an essential tool in the development of prevention and mitigation treatments. The detection limit of an analytical method must be low, because some odorants have low odor thresholds. Alternatively, the odorants must be concentrated prior to analysis. The odorants and their concentrations in a sample will influence the choice of a method of analysis. The sampling approaches described in this section cover the range of simple, rapid field methods for easy practical use through to the very complex instrumentally dependent methods, requiring laboratory analysis.

6. Sensory Odor Analysis

6.1 Characterizing the sensation experienced by inhaling an odorous sample is the object of a sensory odor measurement program. The human body experiences sensations, processes them, and then reacts. The olfactory system senses odor. Sensory analysis is most effective for samples containing complex mixtures of odorants or odorants at concentration levels below detection of an instrumental technique. It also produces simple, useful results that are meaningful to all concerned.

6.1.1 Standardized testing protocols are available for measuring odor intensity (ASTM E544-99), odor thresholds (ASTM E679-91), and the proposed European Union odour testing standard: Determination of Odour Concentration by Dynamic Olfactometry (prEN13724). The latter includes and does not conflict with ASTM E679-91 requirements. prEN13725 exceeds ASTM E679-91 by specifying sampling practices, olfactometer construction and operation, assessor selection, assessor training, assessor certification, data collection, data processing, data reporting, and definitions.

6.2 *Odor Character Descriptors*—Words or phrases are used to represent the quality of the particular odor of concern to a panelist who senses the odor. A difficulty with odor descriptors like “sweet”, “musty”, “sour”, “putrid”, “rotten”, etc. is that different individuals may use a variety of words or phrases to describe the same odor unless “exemplars” (examples of odor descriptors) are used for training of panelists or inspectors. Using what is called a “Hedonic Tone Scale,” provides the panelist with a numbered scalem, (e.g. ‘–10’ for unpleasant to ‘0’ for neutral to +10 for

pleasant). Hedonic Tone is the subjective parameter of odor and is relative to different individuals. This is essentially the basis of Method 5.06-A.

6.3 *Trained Odor Investigators*—An extension of the use of odor descriptors is the odor patrol that utilizes trained odor investigators, i.e., people who have been trained to quantify odor intensities. These people have "calibrated" their noses to certain odor intensities. They are trained to go "on site" and rate the odor intensity on a numeric scale. Some examples of the types of written reports used for record keeping on-site and for citizen odor complaints appear at the end of this section.

6.4 *Scentometer*—A hand-held device used for direct field measurement of dilution-to-threshold (D/T). Varying proportions of ambient (odorous) air, drawn through an activated carbon filter, are introduced to an individual's nose. The ratio of ambient air to filtered air at which the individual detects an odor becomes the D/T. Odor inspectors using this method require training and experience so they can develop confidence in its application. This device has been used successfully by some inspectors in a few states.

6.4.1 The method of producing D/T with a scentometer device consists of mixing two volumes of carbon filtered air with specific volumes of odorous ambient air. Correct application of the D/T method generally requires odor training and experience. Refer to Table 05.06-1.

Table 05.06-1 Dilutions to Threshold (D/T) with the Scentometer[1].

Dilution to Threshold (D/T)	Carbon Filtered Air Volume[2]	Odorous Air Volume[3]	Odorous Air Inlet Size (cm)
2	2	1	1.25
7	2	0.285	0.625
15	2	0.1333	0.46875
31	2	0.0645	0.3125
170	2	0.0118	0.15625
350	2	0.0057	0.03125

6.4.2 Formula for field determination of D/T using the scentometer:

$$D/T = A \div O \qquad \textit{Equation 6.4.2}$$

where:

D/T = field dilution to threshold,
A = carbon filtered air volume, and
O = odorous air volume.

[1] Adapted from Figure 3. Dilutions to Threshold (D/T) with the Scentometer *In* prEN 13725, and Huey, 1960.

[2] Two 1.25-cm diameter holes for the carbon filtered air flow path.

[3] Odorous air volume calculated from the D/T column.

6.5 *Olfactometry*—An olfactometer with an odor panel is another way to conduct a quantitative sensory analysis of odorous air samples. The apparatus presents an air sample containing the odorous mixture to an individual at varying dilutions with odor-free air. The objective is to determine the level of dilution at which the panelist begins to detect an odor. After a series of exposures, results for the odor panel are calculated. and expressed in the form of an odor dilution ratio required for a percentage of the panel (i.e. 50%) to detect the odor.

6.6 *The Butanol Wheel*—The intensity is also an important parameter of odor that can be used to help overcome the difficulties that individuals experience when comparing different odors. The Butanol Wheel is an approach to odor intensity measurement that uses a reference compound, n-butanol, to which odor intensities are compared. In this way, odors can be analyzed so that individuals not subjected to the actual odors can understand the results

6.6.1 The Butanol Wheel is like an olfactometer because it delivers different dilutions of the odorous compound, n-butanol, to sniffing ports. Intensity of an odorous sample is measured by determining at what dilution level of the Butanol Wheel the sample matches the strength of the butanol vapor. An odor panel (group of people, each one exposed to the odor sample and butanol reference independently) is used to make the comparisons. The intensity of the unknown odor is expressed in terms of the calculated dilution of n-butanol vapor to which the odorous sample is equivalent.

6.6.2 The forced choice ascending concentration approach to olfactometry differs from the Butanol Wheel method in that the latter tests the odorous sample at full strength against a series of diluted standards. The former olfactometer method presents dilutions of the odorous sample itself. The two methods complement each other in that results for odor intensity as well as dilution threshold ratio are obtained.

6.7 *Chemical Analyzers and Instruments*—Instruments and methods used to measure odorous compound concentrations include: hand-held reactive absorbent tubes, available for ammonia and hydrogen sulfide; single compound analyzers, such as a hydrogen sulfide (H_2S) meter, that measures one analyte; multiple compound analyzers, like a gas chromatograph (GC), for which specific detectors that are sensitive to certain types of compounds are required. When the compounds are unknown or the mixture is complex, then a mass spectrometer detector and an electronic library of compounds is necessary. The latter is so expensive and sophisticated that it is usually reserved

for a research setting, rather than for routine monitoring.

7. Summary of Test Methods

7.1 *Method 05.06-A Field Assessment of Compost Odor*—This test was designed to be performed in conjunction with *Method 05.03-A Field Assessment of Compost Color*. Compost odor is a quick test used for field diagnostics to aid in approximating compost stability and maturity. The odor of a representative sample is compared to those in the compost odor assessment chart, five classes of indicative odors.

7.1.1 A sample odor is classified by assigning a value from one to five. The table odor value is matched to that most closely resembling compost odor. A table odor value of one to five , i.e., no odor to raw material odor, is assigned to the compost sample.

7.1.2 *Method 05.06-B Field Sampling of Biofilter Odor Emissions*—This test was designed to measure the effectiveness of biofilters in reducing odor from composting process air streams. Biofilter air emission samples are collected at the biofilter surface as off-gas is released to atmosphere. Samples are preserved in sealed Tedlar® bags.

7.1.3 Test samples are prepared by dispersing the off-gas in odor-free air. Known dilution levels of the off-gas are compared by panelists to two blank samples. Each panelist starts at the highest dilution level, (e.g., two or three dilutions above the estimated detection threshold), and indicates which of the three samples is different from the other two.

7.1.4 Individual best-estimate values of the threshold are derived from the pattern of correct/incorrect responses produced separately by each panelist. Group thresholds are derived by geometrical averaging of the individual best-estimate thresholds.

8. Significance and Use

8.1 The malodorous compounds (odorants) associated with biosolids, manures, and other organic materials are the volatile emissions generated from the chemical and microbial decomposition of organic nutrients. When inhaled, these odorants interact with the odor sensing apparatus (olfactory system) and the person perceives odor.

8.2 Individual sensitivity to the quality and intensity of an odorant can vary significantly, and this variability accounts for the difference in sensory and physical responses experienced by individuals who inhale the same amounts and types of compounds. This distinction between “odor”, which is a sensation, and “odorant”, which is a volatile chemical compound, is important for everyone who deals with the odor issue to recognize. When odorants are emitted into the air, individuals may or may not perceive an odor. Emission, transport and perception are the three conditions necessary to create malodorous situations (Table 05.06-2).

8.3 Many compounds are intense and have odor thresholds in the parts per billion (ppb) concentration ranges. When people perceive what they regard as unacceptable amounts or types of odor, odorous emissions can become an *odor problem*.

8.4 Odor can be an indication of unstable compost and improperly prepared compost. This is especially the case if offensive odors like those produced from hydrogen sulfide, ammonia, and short chain fatty acids are perceived. This can result if feedstock is exposed to prolonged periods of anaerobic conditions during the composting process and when finished stable compost is handled in a manner that promotes anaerobosis.

Table 05.06-2 Basic conditions associated with malodorous situations.

EMISSION	Presence of an odorous volatile chemical, odorant
TRANSPORT	Topographic and atmospheric conditions conducive to transport of the odorant with minimal dilution
PERCEPTION	People are present and they perceive odor

8.5 *Method 05.06-A Field Assessment of Compost Odor*—This test is appropriate for use as a K-12 teaching-aid. The method was devised by E&A Environmental Consultants, Inc. to be performed in conjunction with Method 05.03-A.

8.6 *Method 05.06-B Field Sampling of Biofilter Odor Emissions*—Sensory thresholds are used to determine the potential of biofilters to emit odorous gas that is offensive, or that exceeds an air pollution regulatory limit.

9. Interference and Limitations

9.1 *Method 05.06-A Field Assessment of Compost Odor*—Odor should not be used as the sole criteria for inspecting and rejecting loads of compost delivered to a site.

9.1.1 Odor should only be used to indicate compost maturity in those instances when the producer is experienced and the feedstock and composting technology are known.

9.2 *Method 05.06-B Field Sampling of Biofilter Odor Emissions*—Great care must be taken in collecting biofilter off-gas samples to avoid dilution with ambient

air, and to avoid accelerating process airflow through the biofilter.

10. Sample Handling

10.1 *Method 05.06-A Field Assessment of Compost Odor*—As-received, unsieved compost sample material placed in a sample storage bag is required for this test, (e.g., a 1-qt plastic Ziploc® storage container).

10.2 *Method 05.06-B Field Sampling of Biofilter Odor Emissions*—Biofilter off-gas samples are collected and contained in sealed bags. Samples should not be kept more than 24 h before use in ASTM E679–91.

Test Method: Odor. Field Assessment of Compost Odor						**Units:** unitless		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.06-A		05.06-A		05.06-A		

05.06-A FIELD ASSESSMENT OF COMPOST ODOR

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

NOTE 1A—This test is appropriate for use as a teaching-aid, i.e., K-12. The method was devised by E&A Environmental Consultants, Inc. to be performed in conjunction with Method 05.03-A Field Assessment of Compost Color.

11. Apparatus for Method A

11.1 *None required.*

12. Reagents and Materials for Method A

12.1 *Sample containers*—plastic containers or bags with seal, (e.g., 1-qt Ziploc® storage container).

13. Procedure for Method A

13.1 Open a sample container of the compost sample.

13.2 Place your nose near the container opening.

13.3 Smell the compost.

13.4 Assess and rate the odor following the descriptions listed in Table 05.06-A1.

13.5 Record the odor rating and characteristic for the compost sample, (1 through 5, from Table 05.06-A1).

Table 05.06-A1 Odor assessment and rating table.

Odor Rating	*Odor Characteristic*
1	Earthy, soil-like, no odor
2	Moldy, musty, mildew, swampy
3	Fruity, sweet, black licorice, slight pine, slight ammonia, tobacco, burnt odor
4	Sour, rotting grass, manure, sour milk, vinegar, strong ammonia, turpentine, urine
5	Fresh yard debris, wet leaves, hay, strong pine odor

Test Method: Odor. Field Sampling of Biofilter Odor Emissions						**Units:** unitless		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
			05.06-B					

05.06-B FIELD SAMPLING OF BIOFILTER ODOR EMISSIONS

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

NOTE 1B—This test is intended for use in conjunction with ASTM Test Method E 679–91 Standard Practice for Determination of Odor and Taste Thresholds by a Forced-Choice Ascending Concentration Series Method of Limits.

14. Apparatus for Method B

14.1 *Funnel*—Teflon funnel attached to the gas-sampling end of the flexible tubing.

14.2 *Hood*—Box-like container to prevent ambient air dilution of biofilter surface sample.

14.3 *Tubing*—Flexible Teflon tubing rated for vacuum service to connect the vacuum box and vacuum pump, and to extend from the vacuum box air header to the sampling funnel. A quick-disconnect can be used for the vacuum tubing connection to the vacuum box from the vacuum pump, to ease portability of the vacuum box.

14.4 *Vacuum pump*

14.5 *Vacuum box*—Rigid, leak-proof box, such as a sturdy picnic box, connected to the vacuum pump via a quick-disconnect and flexible tubing, and equipped with a vacuum gage and shut-off valve, and with ports inside the box to attach four Tedlar® bags, with external valving to isolate each bag.

15. Reagents and Materials for Method B

15.1 *Bags*—Nine , Tedlar® 1-L capacity.

16. Procedure for Method B

16.1 *Biofilter Off-gas Collection*—Assemble the vacuum box and place four Tedlar® bags inside connected to the respective air nipples. Close the valves between the sample header and each port. Turn on the vacuum pump to evacuate the vacuum box. When a vacuum of approximately 88,000 pascals (26 in. of Hg) has been attained inside the vacuum box, close the shut-off valve to maintain the vacuum inside the box. Disconnect the tubing from the vacuum box to the vacuum pump.

16.2 Take the air-evacuated vacuum box containing the Tedlar® bags to each of three predetermined locations over the biofilter surface. Place a wind hood over the sampling point. Place the sampling funnel inside the wind hood at or near the biofilter surface. Open the valve for the first Tedlar® bag to evacuate air from the collection system and replace it with odorous air. Then open the valve for each remaining Tedlar® bag in turn to collect three samples of off-gas. Repeat this procedure to collect samples from a total of three different locations.

16.3 Care should be taken during the above procedure to avoid compressing the biofilter surface. A full sheet of plywood (4×8) can be used as a walking surface to distribute the load and minimize biofilter media compression.

16.4 The three filled Tedlar® sample bags from each of the three sample locations should be sealed and labeled, and stored for no more than 24 h before use in the odor panel test, ASTM Method E 679–91.

17. Determination of Odor Threshold

17.1 Odor samples should be evaluated in accordance with ASTM Method E 679–91 (Reapproved 1997) Standard Practice for Determination of Odor and Taste Thresholds by a Forced-Choice Ascending Concentration Series Method of Limits.

05.06 METHODS SUMMARY

18. Report

18.1 *Method 05.06-A Field Assessment of Compost Odor:*

18.1.1 *Verify Field Assessments*—It is highly recommended that a sample is submitted for the following laboratory analysis: C:N ratio, NH_4:NO_3 ratio, respirometry test results and stability rating, and biological assay screening tests and other maturity indicators. Refer to Method 05.02-G CCQC Maturity Index.

18.1.2 *Odor*—Report the sample odor rating and characteristic for each sample. Refer to Table 05.06-A1 Odor assessment and rating table.

18.1.3 Report the type of material analyzed, (e.g., compost, feedstocks, etc.), source materials, (e.g., municipal solid waste, biosolids, manures, yard waste, etc.).

18.2 *Method 05.06-B Field Sampling of Biofilter Odor Emissions*:

18.2.1 Report sample odor Dilutions to Threshold (D/T) as required per directions found in ASTM test method E 679–91.

19. Precision and Bias

19.1 *Method 05.06-A for Field Assessment of Compost Odor*—The precision and bias of this test has not been determined. Data are being sought for use in developing a precision and bias statement.

19.2 *Method 05.06-B Field Sampling of Biofilter Odor Emissions*—The precision and bias of this test has not been determined. Data are being sought for use in developing a precision and bias statement.

20. Keywords

20.1 air pollution; ascending method of limits; color; evaluation, humus; maturity; odor; panel; sensory threshold; stability

APPENDIX I TO 05.06—EXAMPLE OF PERFORMANCE STANDARDS FOR ODOROUS EMISSIONS FROM A PERMANENT CONSTRUCTED FACILITY

ADAPTED FROM—Compost Site Conditional Use Permit, Courtesy of St. Croix Sensory, Inc.

21. Odor Testing Practice

21.1 This odor testing practice references the odor intensity of the ambient air to an *Odor Intensity Referencing Scale*, (OIRS).

21.2 The odor of the ambient air is matched (ignoring differences in odor quality) against the OIRS (see Section B in the following section) by trained inspectors. The inspector reports that point, or in between points, on the reference scale which, in her (his) opinion, matches the odor intensity of the ambient air.

21.3 The procedure followed for field odor testing is in accordance with Procedure B - Static-Scale Method of ASTM E-544, except for the following adaptations:

21.3.1 The geometric progression scale ratio = 3.

21.3.2 Use screw-cap containers for reference concentrations of butanol in water.

21.3.3 Inspectors may memorize the OIRS.

21.3.4 Inspectors may use a charcoal filter, breathing mask to avoid olfactory adaption (fatigue) in the ambient air.

21.3.5 Inspectors sniff ambient air and match its intensity to the reference scale.

21.3.6 Inspectors breathe charcoal filtered air for three minutes in between snifffings of ambient air.

21.3.7 Odorous air sampling shall be performed upon the complainant's property. The complainant shall not accompany the inspector and results shall be released after a written report is filed. The inspector shall not conduct the odorous air sampling if the complainant is present.

21.3.8 The inspector shall also sample the ambient air immediately upwind from the compost site to determine the presence and level of any odors entering the site from other sources. These records and observations shall be a part of the written report

21.3.9 The Odor Intensity Referencing Scale (OIRS) will use numbers and descriptions corresponding to butanol concentrations as indicated in Table 05.06a-1.

Table 05.06a-1 Odor Intensity Referencing Scale, OIRS.

Number Scale	*Category Description*	*N-Butanol (ppm) in air/ in water*
0	No Odor	0/ 0
1	Very Faint	25/ 250
2	Faint	75/ 750
3	Distinct, Noticeable	225/ 2250
4	Strong	675/ 6750
5	Very Strong	2025/ 20250

21.3.10 Reasonable operating conditions will allow for a designated number, X, or fewer recorded sniffings by an inspector of the ambient air over a period of Y minutes with a geometric average OIRS value of:

21.3.10.1 Three or greater if there is a permanent residence upon the property, or,

21.3.10.2 Four or greater if the property does not contain a permanent residence.

APPENDIX II TO 05.06—ODOR NOTIFICATION FORM

NOTE—The purpose of this form is to identify odors than can potentially migrate from industrial parks and to communicate those observations to the respective facilities so they can take preventive or remediative action.

Notifier/Phone: _____

Odor Date/Time: _____

Temperature: _____

Strength of Odor: ◘ weak ◘ moderate ◘ strong

Wind speed/direction: _____

Location of Odor: _____

Source	***Odor Type Detected per Source***			
Wastewater Treatment	1° Treatment	2° Treatment	Biosolids	Other _____
Incinerator	Smoke	Ash	Hopper Juice	Other _____
Cover Technologies	Leaf/Earthy	Yard Waste	Raw Paper Sludge	Other _____
Landfill Gas	Natural Gas	Other _____		
Landfill	Sludge	Other _____		
Compost	Compost	Other _____		
Waste Stream	Sludge	Ammonia	Other _____	
Street Biofilter	Chemical	Sewage	Other _____	

Odor Descriptors: (check all applicable)

- ◘ sewer
- ◘ rotten eggs
- ◘ smoky
- ◘ putrid foul decayed
- ◘ garbage
- ◘ musty earthy
- ◘ chemical fecal (like manure)
- ◘ burnt
- ◘ other _____

Source contacted: _____

Source copied: _____

Message left: _____

Location of Odor: _____

Senior Operator: _____

Odor confirmed by Sr. Operator? ◘ yes ◘ no

Comments:

APPENDIX III TO 05.06—RESIDENT ODOR COMPLAINT FORM

Date/Time of Odor: ◘ AM; ◘ PM _____

Wind speed/direction: _____

Air Temperature: _____

Relative Humidity: _____

Weather Conditions: _____

Senior Operator: _____

Odor Descriptors: (check all applicable)

◘ sewer	◘ rotten eggs	◘ smoky
◘ putrid foul decayed	◘ garbage	◘ musty earthy
◘ chemical fecal (like manure)	◘ burnt	◘ other _____

RESIDENT INFORMATION

Name: _____

Address: _____

City: _____

Zip Code: _____ *Telephone #:* _____

Odor Description: *(indicate all applicable)*	◘ sewer ◘ garbage truck	◘ putrid foul decayed ◘ rotten eggs	◘ chemical ◘ burnt smoky	◘ fecal (like manure) ◘ musty earthy
Duration / Frequency of Odor:	_____			
Intensity of Odor:	◘ weak	◘ moderate	◘ strong	

SENIOR OPERATION INFORMATION *(Detailed)*:

Test Method: Organic Matter. Three Methods						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.07-A		05.07-A		05.07-A		05.07-A
							05.07-B	05.07-B
05.07-C	05.07-C	05.07-C		05.07-C	05.07-C	05.07-C		05.07-C

05.07 ORGANIC MATTER

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This test covers the determination of organic matter content in compost.

1.1.1 *Method 05.07-A Loss-On-Ignition Organic Matter Method (LOI)*—A direct determination method that indicates organic matter content by quantifying the amount of solid material combusted relative to the original oven dried sample.

1.1.2 *Method 05.07-B Humic Substances*—Proposed Fulvic Acid and Humic Acid Extraction and Characterization.

1.1.3 *Method 05.07-C Calculations for Organic Matter Decomposition*—This method covers the determination of organic matter decomposition of batch process compost. The protocol is not suitable for use with continuous flow-type composting technologies. This approach to measuring compost stability status is not strongly recommended. No practical test method has been developed, except on biosolids where US EPA CFR Chapter 40 Part 503 references a volatile solids reduction test for biosolids.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Review of Organic Matter

2.1 *Background*—Organic matter is an important reservoir of carbon and a dynamic component of soil and the carbon cycle. It impacts the physical, chemical and biological properties of a soil. Addition of organic matter to soil alters its physical characteristics by changing plant available soil water retention, infiltration, drainage and aeration. Structural parameters are optimized for plant growth by lowering soil bulk density, increasing water holding capacity and aeration. Chemically, the soil nutrient status or nutrient carrying capacity is enhanced by organic matter. Biologically, an enhanced soil organic matter fraction serves as a rich nutrient reservoir and energy source for beneficial microbes.

2.2 *Source*—Soil organic matter content can be increased through frequently repeated applications of compost. Organic matter test determinations will correspond to a compost's stability status and aid in defining the commercial value of a compost relative to its organic matter content.

COMMENT—An organic matter management plan would become practical with the development of compost organic matter test method that could be used to help predict the outcome of applying compost to soil. Present methods simply determine the concentration of organic matter in compost. Knowledge of compost organic matter content does not relate directly to a percentage of soil organic matter after compost is applied to the soil. Factors that clearly alter the organic matter concentration in soil include moisture, temperature, and aeration. An organic matter management plan considers the organic matter content of a compost as one parameter to calculate a compost application rate and frequency for a given soil, to raise that soil's organic matter content to a predetermined target level. This requires identification of a common test method (or suite test methods) for both compost and soil.

CAUTION—Careful attention must be given to historical reports to differentiate references of organic matter measurements versus determinations of total organic carbon. Total organic carbon is used when calculating a C:N ratio. Organic matter contains a number of components in addition to carbon, including nitrogen, sulfur, oxygen, and various micronutrients.

2.3 *Occurrence*—Organic matter is the sum of substances containing organic carbon (Schnitzer,

1991), and is defined as the total organic components in soil including undecayed plant and animal tissues, their partial decomposition products, and the soil biomass exclusive of the macrofauna and macroflora (Vaughan et al., 1985). Organic matter or humus consists of two broad categories known as non-humic and humic substances. The non-humic groups are simple compounds such as carbohydrates, aliphatic and aromatic hydrocarbons, amino acids, ethylene, and hydrogen sulfide that are easily degraded by soil organisms. In contrast, the humic fraction is made up of complex organic molecules, usually formed as byproducts of decomposition and resistant to further degradation. The two stable components of humic substances that play a dominant role in soil physical properties are humic and fulvic acids. These weak acids are also present in organic waste and are suggested to be chemically and structurally similar to humic substances in soil (Sposito et al., 1982).

2.3.1 Organic matter acts as both a sink and source in the soil system. It is a large pool for storage of nitrogen, phosphorus, and sulfur, and can supply nutrients for plant growth. Mineralization of organic matter by microorganisms releases nitrogen, phosphorus, and sulfur to plants. The mineralization of organic matter in grassland soils has contributed to much of the nitrogen and phosphorus nutrition of crops (Tiessen and Stewart, 1983). The negatively charged carboxylic and phenolic functional groups of organic matter produce a high cation exchange capacity relative to other soil fractions (Bohn et al., 1985; McBride, 1994). The functional groups attract metals, metal oxides, hydroxides, and clay minerals to reduce trace metal solubility (Emmerich et al., 1982a; Emmerich et al., 1982b; Leita and DeNobili, 1991).

2.3.2 Organic matter can be partitioned into fresh, slightly humified, and humified state of decomposition (Conti et al., 1993). The humified organic matter is chemically stable and mature (that is, free of organic phytotoxins). Humified organic matter releases nutrients slowly, similarly to a slow release fertilizer (Chen and Avnimelech, 1986). The rate of nutrient release varies with soil physical and chemical properties, climate, microbial population, and the degree of maturity.

2.4 *Nitrogen and Carbon Dynamics*—Nutrient cycling involves immobilization and mineralization driven by microbial activity (Duxbury et al., 1989). Nutrient turnover from labile soil organic matter (which includes soil microbial population) is affected by the carbon supply to heterotrophic microorganisms (Theng et al., 1989). Nitrogen mineralization rates are dependent upon the carbon to nitrogen ratio (C:N ratio).

2.4.1 The dynamic of soil carbon and nitrogen with four cropping systems in agroecosystems was studied by Mazzarino et al. (1993). Sources of carbon and nitrogen additions included tree prunings, corn and bean residues, and inorganic fertilizers. The long-term addition of organic matter in the tree alley cropping treatments increased total and microbial carbon and nitrogen, water-soluble carbon, and soil moisture. Ladd et al. (1977) attempted to partition the mineralization potential from the organic nitrogen component in soil and demonstrated that nitrogen mineralization and availability to crops varies with waste type. Bitzer and Sims (1988) studied nitrogen mineralization in soils amended with poultry manure. They found that organic nitrogen from poultry manure mineralizes rapidly and was even enhanced by the addition of inorganic nitrogen. Rees, *et al.* (1993) studied the influence of the rate and type of manure on nitrogen uptake and uptake efficiency in wheat and barley. They found that nitrogen uptake by barley was increased when inorganic nitrogen fertilizer was added with poultry manure. Bremer and Kessel (1992) reported that 40% of the nitrogen in a lentil green manure was potentially available for plant uptake. Tyson and Cabrera (1993) showed that composted poultry litter mineralized less nitrogen than uncomposted poultry litter, reducing the potential of nitrate pollution. Smith, *et al*. (1993) showed that there was a rapid mineralization of organic nitrogen with treatments of 10 MT A^{-1} alkaline pasteurized sewage sludge.

2.4.2 Changes in organic matter and net mineralization rate are influenced by the cropping system, type of litter, environmental factors, and microbial populations (Van Vuuren et al., 1993; Mazzarino et al., 1993; Rees et al., 1993; Zak et al., 1993). In plant communities dominated by dwarf shrubs, van Vuuren et al. (1993) found that net nitrogen mineralization rates increase with increasing amounts of organic matter and soil nitrogen. When litter was replaced by grass, no clear effect was seen on net nitrogen mineralization rates.

2.4.3 Residue quality is another factor affecting nitrogen turnover (Honeycutt et al., 1993). Two residue qualities of hairy vetch harvested in the fall and spring had different carbon and nitrogen mineralization rates independent of the residue loading rate. Approximately 35% of the added carbon mineralized 30 days after application of the fall vetch, and 17% of added carbon mineralized 30 days after application of spring vetch. The effect was postulated to be due to lignin or hemicellulose contents of the vetch rather than residue nitrogen content or C:N ratio (Honeycutt et al., 1993).

2.5 *Physical Properties*—Soil organic matter influences physical, chemical, and biological properties of the soil. Physical effects of organic matter on soil include improved soil structure, increased aeration, and increased water holding capacity and decreased density. These physical modifications to soil structure modify conditions for root development. Enhanced root development improves water use efficiencies and nutrient uptake.

2.5.1 Most agricultural cropping systems return relatively low amounts of organic matter to soil as crop residues. Soil structure is damaged under continuous cropping systems. Over time, this reduces root penetration and development, and soil aeration. Crop yields are negatively affected by the decreased soil aeration and drainage, due to the depletion of organic matter and increase in soil bulk density. Compost amendments can reverse many negative factors associated with intensive crop production.

2.6 *Organic Matter and Aggregate Sizes*—Soil aggregates are not random assemblages of small particles, but are stabilized aggregates of increasingly larger units that are held together by different organic binding agents. Aggregate formation is a continuum.

2.6.1 Among the physical properties affected by organic matter, the degree of aggregation is fairly well studied (Piccolo and Mbagwu, 1990). Direct correlation were found between total organic matter and aggregate stability (Christenson, 1986). A recent approach in the evaluation of mineralizable organic carbon and nitrogen is to establish aggregate size distribution of organic matter. Some investigators observed that the different size fractions of organic matter are more important to predict organic matter turnover (Cambardella and Elliott, 1992; Elliott, 1986, Janzen et al., 1992).

2.6.2 Organic matter can be fractionated into light and heavy fractions. The light fraction which includes particulate organic matter (POM) is labile, mineralizable, and plays a role in carbon and nitrogen turnovers (Janzen, 1987; Janzen et al., 1992). The light organic matter fraction consists of organic material in various stages of decomposition and has a density of less than 1.6 g cm^{-3} (Janzen, 1987; Janzen et al., 1992; Cambardella, 1994). The relative concentration of carbon and nitrogen in this fraction is high compared to the heavy organic matter fraction (Cambardella et al., 1992; Strickland and Sollins, 1987). Organic matter concentrations may differ within particle size fractions. The enriched labile fraction *(ELF)* of organic matter is used to bind soil particles and form aggregates. As aggregate size increases, the ELF is protected more and more from microbial attack and remains in the soil for longer periods of time unless mechanical disturbances occur (Cambardella, 1994). Particulate organic matter *(POM)* is the organic matter fraction embedded in aggregate structure that is more exposed to microbial attack than ELF. The degree of physical occlusion (i.e. POM occludes ELF) can limit the physical accessibility of carbon and nitrogen sources to microbes. Particulate organic matter which consists primarily of decaying plant roots, is much lighter than ELF and is highly influenced by soil management (Cambardella and Elliott, 1993; Wander et al., 1994). The POM may be a major pool for supplying plant available nutrients. The heavy fraction which can be separated by density or sieving, is mostly associated with the clay fraction (Cambardella and Elliott, 1992; Cambardella and Elliott, 1993).

2.6.3 In a recent study, Cambardella and Elliott (1994) found high organic carbon and nitrogen associated with macroaggregates. Further, they found that 18% of the total carbon and 25% of the total nitrogen in no-till soil was associated with fine-silt size particles having a density of 2.07 to 2.22 g cm^{-3}. Piccolo and Mbagwu (1990) studied the effect of organic waste (pig slurry, cattle slurry, and sewage sludge) amendments to evaluate their influence on aggregate stability and molecular sizes of humic substances. They separated the surface soil into microaggregates of sizes 250-125, 125-50, and < 50 μm. The organic waste amendment linked together the fine particles promoting the formation of stable aggregates. Microaggregate stability is well correlated with the humic substance fraction of organic matter (Piccolo and Mbagwu, 1990; Chaney and Benson, 1984).

2.7 *Soil Structure and Stability*—Intensive agricultural management systems that do not return significant quantities of plant residues to a soil cause degradation of soil structure and severe soil erosion (Campbell, 1982; Elliott, 1986). Soil structure is intimately related to soil aggregate stability, which is dependent upon the presence of organic matter and organic binding agents. Organic matter has chemical and biological agents that act to glue soil particles together (Rose, 1991). Proper soil aggregation provides large, structured soil pores. Large aggregates formed in the presence of organic matter are non-capillary pore spaces through which air penetrates and excess water is drained.

2.7.1 Three types of organic binding agents have been classified: i) transient - rapidly decomposable polysaccharide; ii) temporary - roots and fungal hyphae; and iii) persistent - lignin, cellulose, hemicellulose (Tisdall and Oades, 1982). Soil aggregates are categorized into two relative size

classes: i) macroaggregates; and ii) microaggregates. Macroaggregates are bound by temporary binding agents such as roots and fungal hyphae, and may be destroyed with tillage. Microaggregates are bound by persistent organic agents independent of management, and are not destroyed by cultivation (Tisdall and Oades, 1982). Cambardella and Elliott (1993) observed that no-till management can ameliorate the detrimental effects of intensive cultivation by promoting macroaggregate stability and increasing organic carbon and nitrogen accumulation.

2.7.2 The addition of municipal waste to soils decreases soil bulk density (Kreft, 1987; Tester, 1990). The decrease in bulk density is due to both a dilution effect and an increase in non-capillary pore space. In a preliminary analysis, municipal solid waste compost moldboard plowed at 20 cm on a loamy sand soil showed lower bulk density values compared to the un-amended control (Mamo et al., 1993).

2.8 *Water Retention and Infiltration*—Soil organic matter increases soil water holding capacity. This is particularly salient for coarse, well drained soils, where water infiltration rates are high and irrigation is required to maintain viable crop production. Kreft (1987) found an increase in soil moisture on a loamy sand soil with additions of municipal solid waste compost. Plant available water and water available for microorganisms may not rise with additions of MSW compost (Pera et al., 1983). Kreft (1987) and Cook et al., (1994) demonstrated that water retention increases upon the addition of MSW compost, but plant available water for the fine soils did not increase. Turner et al. (1994) reported an increase in water holding capacity of sandy soils amended with MSW compost with no apparent increase in plant available water. Stabilized organic matter in soils can retain up to four times its own weight of water but only about one half of this may be available to plants (Simpson, 1983). This is due in part to the higher water tension of the organic matter and the general increased presence of soluble salts.

2.9 *Heat Retention*—The presence of humic substances with their unique colloidal chemistry gives soil a dark brown color - contributing to higher absorption of radiation. The volumetric heat capacity of organic matter is higher than all other components of the soil with the exception of liquid water.

2.9.1 Organic matter can lower the overall soil thermal conductivity of well to excessively well drained soils and organic matter additions to poorly to very poorly drained soils can increase the overall soil thermal conductivity by enhancing the air capacities of these soils.

2.10 *Variable Rate Compost Applications*—Advantages and disadvantages of compost applications are considered to construct the conceptual model to optimize compost applications to manage spatially variable soil conditions. As soil physical, chemical and biological conditions vary across the landscape, so do the relative benefits of nutrient applications (Malo and Worcester, 1975). Because compost is an expensive, relatively scarce and sometimes toxic soil amendment, it is difficult to justify high rate applications across entire fields and farms. Computer-controlled technologies, global positioning systems (GPS), satellite and low altitude aerial imagery, and geographic information system (GIS) are effective tools for mapping and optimizing variable rate compost applications.

2.11 *Organic Matter Management and Spatial Modeling*—Soil attribute characteristics derived from remotely sensed imagery of bare soil provides high resolution models that accurately express soil texture variability, soil drainage patterns, soil organic matter variations and other soil attributes that influence soil water-holding characteristics. A soil organic matter management plan focuses on manipulations of soil water-holding characteristics by tailoring compost application rates and frequencies to the natural soil patterns in the farming landscape (i.e., high application rates and frequencies of compost in areas with low organic matter content, and low application rates and frequencies in areas with high organic matter content).

2.11.1 Digital imagery, (e.g., remotely sensed near infra-red [NIR] imagery of bare soil), lends itself to modeling spatial variations in soil parameters and can indicate optimal, suboptimal or possibly inappropriate landscape positions or locations for compost applications within a field (Zheng and Schreier, 1988). For example, the light zones in Fig 05.07-1 (high NIR reflectance) indicate low soil organic matter content, low water-holding capacity and coarse soil texture (Fig 05.07-2). At the opposite end of this scale, dark zones (high NIR absorbance) indicate high soil organic matter content, high water-holding capacity and fine soil texture.

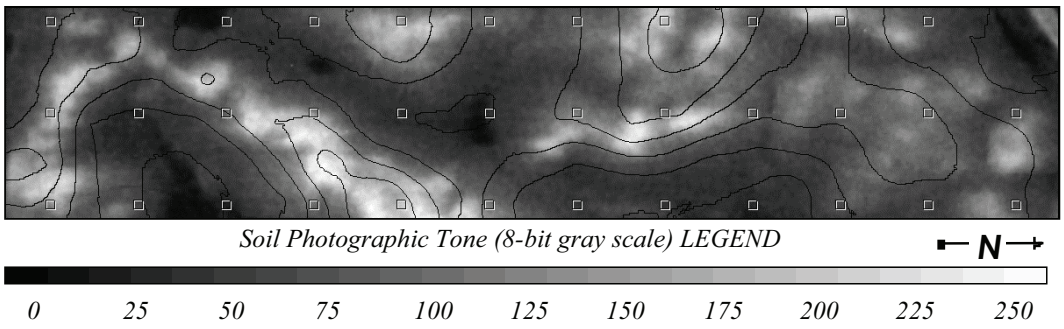

Fig 05.07-1 Gray scale image (8-bit) of a low-altitude color infra-red aerial photograph of bare soil. The uniformly spaced small squares within the image represent soil sample collection points.

ADAPTED FROM—Thompson and Robert, 1995.

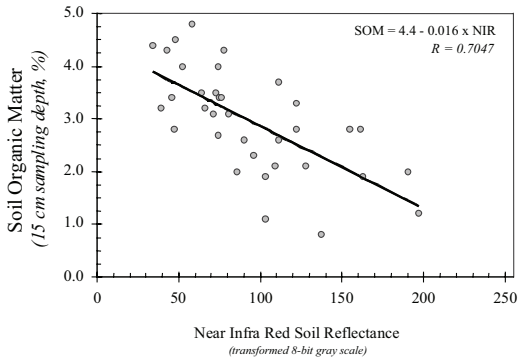

Fig 05.07-2 Soil Organic Matter model used to calibrate a remotely sensed image of bare soil. Calibration technique to transform remotely sensed imagery into spatially variable soil attributes.

2.12 *Practicality of Variable Rate Applications*— Significant research has not yet been conducted to adequately demonstrate the theoretical benefits of variable rate compost applications. Related landscape studies and small plot research on individual soils indicate that appropriately defined compost applications are beneficial and will significantly enhance the productivity of most soils. Throughout the review of organic matter, it is repeatedly reported that compost applications do modify soil physical, chemical and biological characteristics. Extrapolation of these concepts to optimize variable rate applications suggests a viable strategy for efficient and optimized utilization compost products. Bulk application equipment is available that, with minor modifications, will accommodate computer-controlled variable rate compost applications (Fig 05.07-3).

PROVIDED BY—Imagery from Highway Equipment Co., IL (1997).

Fig 05.07-3 Bulk compost spreading equipment can be modified to facilitate variable rate compost applications.

3. Referenced Documents

3.1 *TMECC Methods:*

Method 02.02-A Sample Mixing and Splitting.

Method 02.02-C Man-Made Inert Removal and Classification.

Method 02.02-D Milling and Grinding Samples, Harrison Method, or

Method 02.02-E Milling and Grinding Samples, Munter Method.

Method 02.02-F Modifications for Feedstock Sample Preparation.

Method 03.02-B Milled Material Ignited at 550°C with Inerts Removal.

Method 03.09-A Total Solids and Moisture.

Section 04.01 Organic Carbon.

3.2 *Other References:*

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4. Terminology

4.1 *ash, n*—The inorganic material, or mineral residue of total solids that remains when a compost or feedstock is combusted at 550°C in the presence of excess air; *fixed solids*, % $g.g^{-1}$.

4.2 *biodegradable volatile solids, n*—The organic carbon compounds of total solids that volatilize to carbon dioxide and other gasses when a compost or feedstock is combusted at 550°C in the presence of excess air, % $g.g^{-1}$.

4.3 *compostable, n*—Biodegradable materials that decompose significantly during the retention time of a self-heating composting process; biodegradable materials that readily degrade to carbon dioxide and water when incorporated into a compost pile.

4.4 *fixed solids, n*—The inorganic material, or mineral residue of total solids that remains as ash when a compost or feedstock is combusted at 550°C in the presence of excess air; *Ash*, % $g.g^{-1}$.

4.5 *humic substances, n*—They are complex organic fractions, usually formed as byproducts of decomposition that resist further degradation. Humic

acid, fulvic acid, and humin are humic substances. They are chemically complex substances of high molecular weight, and tend to be amorphous, dark-colored, hydrophilic and acidic. Two stable components of humic substances that play a dominant role in soil physical properties are humic and fulvic acids. These weak acids are also present in organic waste and are suggested to be chemically and structurally similar to humic substances in soil (adapted from Sposito et al., 1982).

4.5.1 *fulvic acids, n (FA)*—fraction of humic substances that solubilize in an alkali solution and is not precipitated by acid. It can form water-soluble complexes at any pH and exhibits a greater affinity for Fe^{3+} and Al^{3+} than other cations. This affinity varies with pH.

4.5.2 *humic acid, n (HA)*—fraction of humic substances that solubilize in dilute alkali conditions and is precipitated by acid. It can form water-soluble complexes at pH's greater than 6.5, but below this pH humic acid is insoluble.

4.5.3 *humin, n*—fraction of humic substance that does not solubilize in either weak acid or alkaline solution.

4.6 *moisture content, n*—The liquid fraction (percentage) of a compost or feedstock that evaporates at 70±5°C, % $g.g^{-1}$.

4.7 *organic carbon, n*—biologically degradable carbon containing compounds found in the soil or compost organic fraction. They originate from sugars, starches, proteins, fats, hemicellulose, cellulose and lignocellulose that are found in composting feedstock and are biologically degraded during composting and curing. Other organic carbon forms that are generally not degraded biologically include petroleum and petroleum byproducts, such as plastics and contaminated oils. They can be degraded by physical means, for example if the temperature is sufficiently high. It does not include inorganic carbonate concretions such as calcium and magnesium carbonates.

4.8 *organic matter fractions, n (e.g., humic substances: fulvic acid; humic acid; and humin)*—complex mixtures of polymeric organic molecules that cannot be separated into homogeneous molecules and cannot be precisely defined in chemical terms. Fraction ratios vary directly with the strength of base and acid employed in the extraction/separation procedure.

4.9 *organic matter, n (OM)*—the sum of solids in compost that contain organic carbon (adapted from Schnitzer, 1991); the total organic components in compost including undecayed plant and animal tissues, their partial decomposition products, and the compost biomass exclusive of living macrofauna and macroflora (adapted from Vaughan et al., 1985).

4.10 *organic matter, n*—the sum of solids in compost that contain organic carbon; the total organic components in compost including undecayed plant and animal tissues, their partial decomposition products, and the compost biomass exclusive of living macrofauna and macroflora.

4.11 *oxidizable carbon, n*—Equivalent to total organic carbon and relative to oxidant employed. Oxidizable carbon is measured by Walkley Black methods devised for use in mineral soils.

4.12 *total solids, n*—The solid fraction of a compost or feedstock that does not evaporate at 70±5°C, which consists of fixed solids, biodegradable volatile solids, and volatile solids that are not readily biodegradable, % $g\ g^{-1}$.

4.13 *volatile solids, n*—Materials that volatilize to carbon dioxide and other gasses when a compost or feedstock is combusted at 550°C in the presence of excess air, % $g.g^{-1}$. The sum of biodegradable solids that degrade during composting, non-biodegradable solids and biodegradable solids that do not degrade during the retention time allowed for composting.

5. Summary of Test Methods

5.1 *Organic Matter Determinations*—Identification and development of a suitable extractant or determination method for organic matter is a major research interest among soil scientists. Procedures commonly used are dichromate oxidation, peroxide oxidation, hot alkali extraction, and loss on ignition (LOI).

5.2 *Method 05.07-A Loss-On-Ignition Organic Matter Method (LOI)*—Organic matter content of a compost sample is determined by igniting an oven-dried sample in a muffle furnace at 550°C. The volatilized material is the organic matter fraction and the remaining ash is the mineral fraction.

5.2.1 The LOI method is a direct determination of compost organic matter. The method is rapid, easy, precise and accurate for properly prepared samples. The compost method is based upon methods developed for use with peat and organic soils.

5.2.2 In the interest of improving intra-laboratory precision and to decrease the time required to complete analysis, 550°C was accepted as most appropriate ashing temperature for organic matter determinations on compost and composting feedstock samples.

5.2.2.1 The method adheres to protocols of similar methods provided in ASTM and AOAC: Test Method

C from ASTM D 2974 - 87 (Reapproved 1995). Standard Test Methods for Moisture, Ash, and Organic Matter of Peat and Other Organic Soils; Test Method 967.05 (final action 1967) from AOAC Official Methods of Analysis Organic Matter in Peat (1990).

5.2.2.2 The range of temperatures used for soil organic matter determination varies from 360 in mineral soils up to 750°C for some organic materials. The organic material fraction is volatilized and the mineral fraction is retained as ash. Percent organic matter content is obtained by difference relative to the bulk oven-dried sample.

5.3 *Method 05.07-B Humic Substances - Proposed Fulvic Acid and Humic Acid Extraction and Characterization*—Humic substances are solubilized with a strong base and extracted. Fractionation and purification is performed to separate fulvic acids from humic acids. Determinations are performed with spectrophotometers following the principle that organic matter absorbs in the ultra violet, visible, and especially the infrared regions.

5.3.1 Humic and fulvic acids are soluble in basic media and can be extracted from soil and organic materials using aqueous alkali solutions. Fulvic acids are soluble at all pH ranges, while humic acids are soluble in basic media only.

5.3.2 The reaction below shows the principle behind the extraction.

$$R(COO)_4Ca_2 + Na_4P_2O_7 \rightarrow R(COONa)_4 \text{ (soluble)} + Ca_2P_2O_7 \text{ (precipitate)}$$

where:

R = aliphatic or aromatic carbon chain skeleton.

5.3.3 Humus makes up a large fraction of organic matter and is important in soil ecology, soil fertility and soil structure. Total organic carbon of compost also contains humic substances that include fulvic and humic acids. The proportion of humus within compost increases with compost stability. In general, the relative proportion of humic carbon to the total organic carbon content of organic matter increases as compost stabilizes. This relationship varies with the nature of the raw materials used to form the compost. Raw materials high in lignin usually yield greater amounts of humus than materials low in lignin.

5.3.4 Humic substances may be beneficial to compost, especially if there are high concentrations of heavy metals within the feedstock. This is because humic acids readily form complexes or chelates with metals e.g., Zn, Mn and Fe reducing the concentration of soluble metals in solution.

5.4 *Method 05.07-C Organic Matter Decomposition Calculations*—The organic matter fraction (OM), occasionally referred to as the biodegradable volatile solids fraction (BVS), of total solids diminishes during the composting as a function of controlled biological decomposition. The total solids fraction includes inorganic materials that remain as ash after ignition at 550°C, the volatile solids in feedstock that biodegrade, and volatile inorganic materials remaining in a finished product such as sand, stones, carbonate concretions, plastic, metal and glass. As feedstock products are degraded, they become biologically stable; carbon dioxide and water are byproducts under aerobic conditions while methane is the main byproduct under anaerobic conditions. This test provides a mechanism for tracking the decomposition process by measuring and documenting changes in organic matter content of materials at multiple stages of the composting process.

5.4.1 Organic matter content is determined for the same material at different stages of a batch composting process, from feedstock preparation to screening and packaging. Samples are ashed at 550°C as described in Method 05.07-A LOI Organic Matter, and percent reduction in organic matter content due to decomposition during the composting process is calculated.

6. Interference and Limitations

6.1 Samples high in petroleum based inert material (hard plastics) or inorganic carbon (carbonates) may significantly inflate compost organic matter determinations if organic matter content is approximated solely from carbon content.

6.1.1 Film plastics alone cause less error because of their minor impact on overall sample mass.

6.1.2 It is imperative to measure inert plastic content of a compost with a parallel sample and correct for carbon contributed by petroleum-based plastics.

6.2 *Method 05.07-A Loss-On-Ignition Organic Matter Method (LOI):*

6.2.1 Deviation from the recommended ashing temperature of 550°C will introduce significant error. Lower combustion temperature can produce a significantly lower LOI OM result.

6.3 *Method 05.07-B Humic Substances - Proposed Fulvic Acid and Humic Acid Extraction and Characterization*—Alkali solutions employed with this method, namely sodium hydroxide and sodium pyrophosphate cause slight oxidation of organic matter, dissolve cellular components of plant residues and other lignins of organic matter that are not yet humified. This tendency alters the expected value representing humic substances. When sodium pyrophosphate is used as an extractant, removal of

phosphate from extracted organic matter is not practicable and will interfere with the analysis.

6.4 *Method 05.07-C Calculations for Organic Matter Decomposition*—Reduction in organic matter is one of the original test methods used to approximate biosolids stability. The reduction of organic matter in compost is not a stand-alone indicator of compost stability or maturity; other indicators must be considered such as C:N ratio, respirometry, pH, bulk density, ammonium to nitrate ratio, etc.

6.4.1 This protocol was designed for compost samples and accounts for the inert content of compost.

6.4.2 The protocol is valid only in batch composting processes when samples are taken on the same composting materials, after initial screening, in-process, and again before final screening.

6.4.3 This test is not applicable for continuous composting processes. By virtue of the continuous blending and multiple screening steps built into most continuous systems, tracking a batch through the process is not practical and prone to significant systematic error.

7. Sample Handling

7.1 *Method 05.07-A Loss-On-Ignition Organic Matter Method (LOI)*—Compost samples should be air-dried at 36°C and sieved through 9.5-mm sieve. Inert materials, especially plastics and plant debris should be removed. If the sample is high in carbonate, an acid wash treatment may be necessary to remove carbonates.

7.2 *Method 05.07-B Humic Substances - Proposed Fulvic Acid and Humic Acid Extraction and Characterization*—Samples must be air dried at 36°C.

7.3 *Method 05.07-C Calculations for Organic Matter Decomposition*—Follow sample cCollection protocols as described in 02.01 Field Sampling of Compost Materials.

7.4 *Test Sample Aliquot Size:*

7.4.1 *Compost Samples*—150 cm^3;

7.4.2 *In-Process Samples*—250 cm^3; or

7.4.3 *Feedstock Samples*—750 cm^3.

7.5 Prepared samples are air-dried, inerts are separated, the compostable materials are milled to a fine powder (< 0.5 mm) and thoroughly mixed. The milled sample shall not contain materials that are not compostable.

Test Method: Organic Matter. Loss On Ignition Method						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.07-A		05.07-A		05.07-A		05.07-A

05.07-A LOSS ON IGNITION METHOD

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Apparatus for Method A

8.1 *Oven*—forced air, set at 70±5°C.

8.2 *Muffle Furnace*—set at 550°C.

8.3 *Sieves*—1-mm stainless mesh sieve.

8.4 *Analytical Balance*—accurate to ± 1.0 mg (e.g., Mettler instruments, or equal).

8.5 *Sample Containers*

8.5.1 *Crucibles (for small sample aliquots)*—ceramic, carbon free (alundum, zircon, or equal).

8.5.2 *Beaker (for large sample aliquots)*—150 mL, Pyrex or equivalent (optional, if larger sample size is preferred).

8.6 *Desiccator*—equipped with calcium chloride as a desiccant (Fisher Scientific, or equal).

9. Reagents and Materials for Method A

9.1 *Hydrochloric Acid*—0.05 *N* HCl.

10. Procedure for Method A

10.1 Oven dry a 10-g compost sample in a forced-air oven set at 70±5°C until sample weight change diminishes to nil, approximately 2 h for air-dried samples and up to 24 h for as-received moist material.

NOTE 1A—Use a larger sample (approximately 100 cm^3) if within sample heterogeneity is significant. This will minimize error associated with sample heterogeneity.

10.2 Cool the oven-dried sample in a desiccator and record the oven dry weight, dw (±0.001 g).

10.3 Remove carbonates by wetting the sample with excess 0.05 *N* HCl. Add acid until foaming ceases.

10.3.1 Dilute the excess acid with distilled water.

10.3.2 Drive off excess moisture from the carbonate-free sample aliquot by oven-drying at 75ºC until weight change due to moisture loss diminishes to nil. Measure and record the oven-dry weight of the sample aliquot.

10.4 Place the sample in a muffle furnace. Slowly ramp the furnace temperature to 550°C. Combust the sample at 550°C for 2 h and then slowly ramp the furnace temperature down to approximately 200°C.

10.5 Remove the ashed samples from the furnace, transfer them to a desiccator and allow them to cool to ambient laboratory temperature.

10.6 Measure and record net ashed weight, AshW (±0.001 g) of each sample.

11. Calculations for Method A

11.1 *Organic matter using Loss On Ignition:*

$$OM = (1 - AshW \div dw) \times 100 \qquad \textit{Equation 11.1}$$

where:

OM = percent LOI organic matter, %.

AshW = sample net weight after ignition at 550°C, g, and

dw = sample net weight after drying at 70±5°C before ignition, g.

Test Method: Organic Matter. Humic Substances							**Units:** mg kg^{-1}	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.07-B	05.07-B	05.07-B				05.07-B

05.07-B HUMIC SUBSTANCES - PROPOSED FULVIC ACID AND HUMIC ACID EXTRACTION AND CHARACTERIZATION

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

12. Apparatus for Method B

12.1 *Drying Oven*—forced air, vented drying set at 70±5°C.

12.2 *Sieves*—4-mm and 6.3-mm mesh plastic sieves.

12.3 *Mechanical Shaker*—reciprocal or equivalent, (e.g., Tyler Model RX-86).

12.4 *Sample Bottles*—200-mL, polypropylene.

12.5 *Centrifuge*—capable of 20,000 *g*.

12.6 *Spectrophotometer*—double beam, Perkin-Elmer or equivalent.

12.7 *Infrared Spectrometer*—Perkin-Elmer or equivalent.

13. Reagents and Materials for Method B

13.1 *Water*—deionized, minimum resistivity 17 MΩ·cm minimum standard.

13.2 *Sodium Hydroxide*—0.1 *N* NaOH; or *Sodium Pyrophosphate*—0.1 *N* $Na_4P_2O_7$.

13.3 *Hydrochloric Acid*—0.05 *N* and 2.0 *N* HCl.

13.4 *Sulfuric Acid*—0.05 *N* H_2SO_4.

13.5 *Cation Exchange Resin*—Amberlite IR-20 or Dowex -50 hydrogen form.

13.6 *Sodium Bicarbonate*—0.05 *N* $NaHCO_3$.

13.7 *Potassium Bromide*—KBr, spectroscopic purity.

14. Procedure for Method B

14.1 *Extraction:*

14.1.1 Leach sample with excess 0.05 *N* HCl to remove carbonates until foaming ceases.

14.1.2 Decant excess acid and wash residue with distilled water.

14.1.3 Air-dry the sample and transfer 10 g of treated sample into 200-mL polypropylene flask.

14.1.4 Add 100 mL of 0.1 *N* NaOH.

14.1.5 Replace headspace air in the flask with N_2 gas, stopper and shake flask for 24 h.

14.1.6 Centrifuge mixture at 10,000 revolutions per min for 10 min.

14.1.7 Decant supernatant into polypropylene container.

14.1.8 Repeat steps 14.1.4 through 14.1.7 two or three times [2× - 3×].

14.2 *Fractionation:*

14.2.1 Suspend the residue in 50 mL of distilled water.

14.2.2 Collect washing in same polypropylene container used in step 14.1.7.

14.2.3 Acidify alkaline extract to pH 2 with 2 *N* HCl, leave extract at room temperature (25°C) for 24 h.

14.2.4 Separate soluble material by centrifugation, centrifuge mixture at 10,000 revolutions per min for 10 min.

NOTE 1B—The soluble material contains fulvic acid (FA), and coagulated contains humic acid (HA). Centrifugation separates supernatant from precipitate.

14.2.5 Freeze dry both fractions.

14.3 *Purification of Fulvic Acid (FA):*

14.3.1 Apply aqueous solution of FA 2× to 3× in succession over hydrogen form resin.

14.3.2 Pass 1 *N* NaOH through resin and collect elute.

14.3.3 Freeze dry residue.

14.4 *Purification of Humic Acid (HA):*

14.4.1 Weigh 1 g of HA in polypropylene bottle; add 100 mL of HCl-HF to bottle; shake mixture for 24 h at 25°C; filter extract through sieve.

14.4.2 Repeat step 11.4.1 3× or 4×.

14.4.3 Wash residue with distilled water and dry.

14.5 *Absorption Method for Characterization of Humic Materials (HA or FA):*

14.5.1 Dissolve 2 to 4 mg of FA or HA in 10 mL of 0.05 *N* $NaHCO_3$.

NOTE 2D—pH should be near 8.0.

14.5.2 Measure absorption at 465 and 665 nm.

NOTE 3D—Use 0.05 *N* $NaHCO_3$ in the reference cell.

14.5.3 Obtain ratio of absorption, E_4/E_6.

14.6 *Infrared Spectrometry for Characterization of Humic Materials (HA or FA):*

14.6.1 Mix 1.0 mg of FA or HA with 400 mg of dry KBr pellets.

14.6.2 Press into suitable die under vacuum at pressure of 7,500 kg cm^{-2} for 20 min.

14.6.3 Measure frequency bands of functional groups.

Test Method: Organic Matter. Calculations for Organic Matter Decomposition						**Units:** % g g^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
05.07-C	05.07-C	05.07-C		05.07-C	05.07-C	05.07-C		05.07-C

05.07-C CALCULATIONS FOR ORGANIC MATTER DECOMPOSITION

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

15. Apparatus for Method C

15.1 *Balance*—analytical, with accuracy of ±0.001 g.

15.2 *Desiccator Cabinet*—vacuum with desiccant tray containing a color indicator of moisture concentration or an instrument indicator.

15.3 *Drying Ovens*—two, forced-air, vented, set at 70±5°C and 36°C.

15.4 *Sample Containers*—glass beakers capable of withstanding temperatures above 550°C, (e.g., Pyrex, etc.); use 150-mL crucibles or beakers with compost samples, and 500-mL beakers with in-process and feedstock samples.

15.5 *Furnace*—forced air muffle, set at 550°C.

15.6 *Mill or Grinder*—capable of milling feedstocks to a fine power, i.e., particle size of <0.5-mm.

15.7 *Sieve*—4-mm mesh, plastic or stainless steel, approximately 30-cm diameter, with capture pan.

15.8 *Watch Glass*—5-cm (2-in.) diameter for 150-mL beakers, and 10-cm (4-in.) diameter for 500-mL beakers.

16. Reagents and Materials for Method C

16.1 *None required.*

17. Procedure for Method C

17.1 *Sample Aliquot Preparation:*

17.1.1 Dry sample aliquots in a forced-air, vented oven until weight change due to moisture loss diminishes to nil:

17.1.1.1 *Compost Samples*—air dry a 150 cm^3 sample aliquot at 36°C;

17.1.1.2 *In-Process Samples*—air dry a 250 cm^3 sample aliquot at 36°C; or

17.1.1.3 *Feedstock Samples*—oven dry a 750 cm^3 sample aliquot at 70±5°C.

17.1.2 Separate the sample into two size fractions with the 4-mm sieve. Gently rub as much material as practical through the 4-mm sieve. Retain each size fraction for further processing.

NOTE 1A—Inert materials that adhere to aggregates of composted particles are more easily separated when samples are air-dried rather than oven-dried. Oven-drying often causes the fragments to strongly adhere, making the segregation process very difficult.

17.1.3 Spread the >4-mm sample onto a clean laboratory tray. Separate the non-compostable materials from the compostable materials. Non-compostable materials do not readily humify. Retain all compostable and non-compostable material separately for further processing.

17.1.4 Recombine the >4-mm compostable fraction with the <4-mm fraction. Grind or mill the recombined compostable fraction to a fine powder (<0.5 mm).

17.2 *Preparation of Evaporating Dish:*

17.2.1 Heat the clean crucibles or beakers to 105°C for approximately 1 h to drive off all hygroscopic moisture.

17.2.2 Place heated beakers or crucibles in a desiccator cabinet to cool to ambient laboratory temperature.

17.2.3 Weigh the crucibles or beakers and record the dry tare weights immediately prior to use.

17.3 *Oven Dry Each Fraction:*

17.3.1 Oven dry the milled compostable fraction at 70±5°C in a forced-air oven for 18 h to 24 h, until weight change diminishes to nil. Cool the sample to ambient laboratory temperature in a desiccator cabinet. Record the oven dry weight, i.e., mass of the compostable fraction solids (S_C).

17.3.2 Oven dry the non-compostable fraction at 70±5°C as described above and obtain the mass of non-compostable solids (S_N).

17.4 *Organic Matter Content:*

17.4.1 *Compostable Fraction Test Aliquot*—Transfer a representative aliquot of the milled compostable fraction to a beaker or crucible:

17.4.1.1 *Compost Test Aliquot Size*—50 cm^3 test aliquot;

17.4.1.2 *In-Process Test Aliquot Size*—150 cm^3 test aliquot; or

17.4.1.3 *Feedstock Test Aliquot Size*—250 cm^3 test aliquot.

17.4.2 Weigh and record the mass of the crucible or beaker, and test aliquot. Subtract the tare weight to determine the mass of the test aliquot (S_A).

17.4.3 Place a watch glass over the mouth of each crucible or beaker; place the crucibles or beakers containing the compostable fraction test sample aliquot in the muffle furnace. Ramp the muffle furnace temperature to 550°C and ash the samples at 550°C for two h.

17.4.4 Allow the muffle furnace to cool by ramping the furnace temperature down to approximately 200°C; transfer the ashed sample crucibles or beakers with watch glass in place to a desiccator and cool to ambient laboratory temperature.

17.4.5 Remove the watch glass. Weigh and record the gross mass of the sample containers and ash; calculate the net weight of ash (A_A) in the test aliquot.

17.5 Track organic matter decomposition through the composting process.

17.5.1 Repeat the determination of organic matter content (Equation 18.1.1) for samples collected at each stage of the composting process and for each batch of interest; repeat steps 17.1 through 17.4 for each organic matter decomposition sample.

18. Calculation for Method C

18.1 *Calculate the organic matter content for each test sample:*

$$OM = V_C \div S_T \times 100 \qquad \textit{Equation 18.1.1}$$

where:

OM = organic matter content, % dw basis,

V_C = compostable material volatilized from the sample, calculated g, and

S_T = combined mass of solids, calculated g,

and:

$$V_C = S_C \times VS_A \qquad \textit{Equation 18.1.2}$$

$$S_T = S_C + S_N \qquad \textit{Equation 18.1.3}$$

$$VS_A = 1 - A_A \div S_A \qquad \textit{Equation 18.1.4}$$

where:

S_A = dry mass of the milled test aliquot before ashing, measured g,

A_A = dry mass of the milled test aliquot after ashing, measured g,

VS_A = fraction of dry solids volatilized from test aliquot, calculated unitless ratio,

S_C = mass of dry solids for the milled compostable fraction of the original sample, dw basis, measured g, and

S_N = mass of dry solids for the non-compostable fraction of the original sample, dw basis, measured g.

18.2 *Calculate organic matter decomposition (D) for finished compost relative to original feedstock blend:*

$$D_3 = C \div F \times 100 \qquad \textit{Equation 18.2}$$

18.3 *Calculate D for in-process material relative to original feedstock blend:*

$$D_2 = P \div F \times 100 \qquad \textit{Equation 18.3}$$

18.4 *Calculate D for finished compost relative to in-process material:*

$$D_1 = C \div P \times 100 \qquad \textit{Equation 18.4}$$

where:

D_1 = stage one decomposition, ratio of organic matter of finished compost versus the organic matter of its in-process material, %,

D_2 = stage two decomposition, ratio of organic matter of in-process material versus the organic matter of its feedstock, %,

D_3 = stage three decomposition, ratio of organic matter of finished compost versus the organic matter of its feedstock, %,

C = organic matter content of finished compost, %,

P = organic matter content of in-process material, %, and

F = organic matter content of original compost feedstock blend, %.

05.07 METHODS SUMMARY

19. Report

19.1 *Method 05.07-A Loss-On-Ignition Organic Matter Method (LOI)*

19.1.1 Report LOI organic matter content as a percentage on an oven-dried basis (70±5°C) with three significant figures.

19.1.2 Report any deviation from the recommended procedures, (e.g., different ashing temperature, etc.).

19.1.3 If present, report the removal of carbonates from sample.

19.2 *Method 05.07-B Humic Substances - Proposed Fulvic Acid and Humic Acid Extraction and Characterization*—Data for samples are reported as ratios to three significant figures.

19.3 *Method 05.07-C Calculation for Organic Matter Decomposition*—Report organic matter decomposition percentage for each stage of the composting process. Report source material, (e.g., municipal solids waste, yard waste, biosolids, etc.), and feedstock blend components.

19.3.1 Never report organic matter decomposition as a stand-alone indicator of compost stability or maturity; other indicators must be considered such as C:N ratio, respirometry, pH, bulk density, ammonium to nitrate ratio, etc.

20. Precision and Accuracy

20.1 *Method 05.07-A Loss-On-Ignition Organic Matter Method (LOI)*—The precision and bias of this test are being determined. Data are being sought for use in developing a precision and bias statement.

20.2 *Method 05.07-B Humic Substances - Proposed Fulvic Acid and Humic Acid Extraction and Characterization*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

20.3 *Method 05.07-C Proposed Calculation for Organic Matter Reduction*—The precision and bias of this test is not determined. Data are being sought for use in developing a precision and bias statement.

21. Keywords

21.1 ash; feedstock; in-process compost; finished compost; humus; humic acid; fulvic acid; humin; organic carbon; organic constituents; organic matter; oxidizable carbon; loss on ignition; LOI; organic matter reduction; ash; solids; total solids; volatile solids;

Test Method: Respirometry. Six Methods						**Units:** see methods		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.08-A		05.08-A		05.08-A		05.08-A
		05.08-B	05.08-B	05.08-B		05.08-B		05.08-B
	05.08-C	05.08-C		05.08-C		05.08-C		05.08-C
		05.08-D	05.08-D	05.08-D		05.08-D		05.08-D
		05.08-E	05.08-E	05.08-E		05.08-E		05.08-E
		05.08-F	05.08-F	05.08-F		05.08-F		05.08-F

05.08 RESPIROMETRY

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This test covers the indirect determination of microbial activity in compost by measuring respiration rates in a compost sample. It is used as an indicator of compost stability.

1.1.1 *Method 05.08-A Specific Oxygen Uptake Rate (SOUR).*

1.1.2 *Method 05.08-B Carbon Dioxide Evolution Rate.*

1.1.3 *Method 05.08-C In-Situ Oxygen Refresh Rate*—Modified after USAEC Report ENAEC-TS-CR-93208.

1.1.4 *Method 05.08-D Dewar Self-Heating Test.*

1.1.5 *Method 05.08-E Solvita® Maturity Index.*

1.1.6 *Method 05.08-F Biologically Available Carbon.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 02.01-A Compost Sampling Principles and Practices

Method 02.01-B Selection of Sampling Locations for Windrows and Piles

Method 02.02-C Man Made Inert Removal and Classification

Method 03.02-C Unmilled Material Ignited at 550°C with Inerts Removal.

Method 03.09 Total Solids and Moisture

Method 04.01 Organic Carbon

Method 05.03-A Field Assessment of Compost Color and Odor

Method 05.07-A Loss on Ignition Organic Matter

2.2 *Apparatus Manuals:*

Cole-Parmer Instrument Company Inc., 7425 N. Oak Park Avenue, Miles, IL 60714 USA; URL: http://www.coleparmer.com.

YSI Incorporated, 1725 Brannum Lane, Yellow Springs, OH 45387 USA; URL: http://www.ysi.com.

Columbus Instruments (MicroOxymax), 950 North Hague Avenue; Columbus, OH 43204-2121 USA; URL: http://www.colinst.com.

2.3 *Other References:*

Carlsbaek, M. and M. Broegger. 1999. Danish soil improvement: new standardized product sheet for compost. Report to Danish EPA. *In* Proceedings of ORBIT99 Organic Recovery & Biological Treatment Symposium. Weimar, Germany.

Iannotti, D. A., M. E. Grebus, B. L. Toth, L. V. Madden, and H. A. J. Hoitink. 1994. Oxygen Respirometry to Assess Stability and Maturity of Composted Municipal Solid Waste. J. Environ. Qual. 23:1177-1183.

Iannotti, D. A., T. Pang, B. L. Toth, D. L. Elwell, H. M. Keener and H. A. J. Hoitink. 1993. A Quantitative Respirometric Method for Monitoring Compost Stability. Compost Science & Utilization 1:52-65.

Jourdan, B. 1982. Standardizing Selected Methods for Determining the Degree of Maturity Decomposition of Municipal Composts. Abfall Wirtschaft Forschungsbericht. Univ. Stuttgart.

Standard Methods for the Examination of Water and Wastewater. 1992. Part 2000, Physical and Aggregate Properties. Method 2710 A. Oxygen-Consumption Rate.

Zibilske, L.M. 1994. Carbon Mineralization. pp. 835-863. In R.W. Weaver (ed.). Methods of Soil Analysis. Part 2. SSSA Book Series 5. SSSA, Madison, WI.

3. Terminology

3.1 *aerobic, adj*—Living or occurring only in the presence of oxygen, (e.g., aerobic bacteria).

3.2 *ammonia (NH_3), n*—A volatile gas that contains nitrogen, ammonia gas.

3.3 *anaerobic, adj*—Living or occurring only in the absence of oxygen, (e.g., anaerobic bacteria).

3.4 *biodegradable volatile solids, n*—The biodegradable portion of total solids that volatilizes to gasses when a compost or feedstock is combusted at 550°C in the presence of excess air, % $g.g^{-1}$.

3.5 *biologically available carbon (BAC)*—CO_2-C as a product of microbial respiration where carbon is the limiting factor of a nutrient-enriched, and physically and chemically optimized compost sample matrix. A parameter proposed to emulate managed agricultural soil conditions.

3.6 *carbon-dioxide evolution, n*—The amount of CO_2 gas generated from the decomposition of organic matter during composting and detected in the headspace as described in Methods 05.08-C and 05.08-E. Determinations of the rate of decomposition as indicated by CO_2 evolution from a compost sample is a reliable means to assess compost stability and one of the indicators of compost maturity. Like other bioassay methods, respirometry can be used as a screening tool to indirectly assess the relative phytotoxicity of compost products.

3.7 *decomposition, n—Biological,* The act or result of decomposing; disintegration and breakdown or decay of organic materials into simpler compounds. *Chemistry,* Separation into constituents by chemical reaction.

3.8 *Dewar vessel*—a super-insulated vessel, invented by Sir James Dewar in 1893. The vacuum lined vessel was intended to keep cool (or hot) materials in a stable state. It was Bernd Jourdan (1982), working at the Institute of Wastewater Management of the University of Stuttgart who first applied the vessel to evaluating compost maturity and self-heating.

3.9 *facultative anaerobes, adj*—Bacteria that are capable of functioning with or without oxygen.

3.10 *maturity index, n*—A rating system devised to categorize compost relative to aging; it is based upon three or more relevant parameters as determined from compost analytical data, (e.g., carbon:nitrogen ratio; respiration rate; and ammonium:nitrate ratio, etc.).

3.11 *respiration, n*—An energy generating process by which an inorganic molecule such as O_2, NO_3, SO_4 or CO_2 is reduced through a series of metabolic steps to form water (H_2O), diatomic nitrogen (N_2), hydrogen sulphide (H_2S) or methane (CH_4). The reducing agent or substance that is first oxidized can be either an organic, (e.g., glucose), or inorganic, (e.g., NH_4Cl), compound. This process occurs within the mitochondria of living cells and in various microorganisms. At the mitochondria of living cells and in heterotrophic microorganisms organic molecules are the energy source and O_2 is reduced. Anaerobic heterotrophs use organic molecules as their energy source, but reduce nitrate and sulfate. Aerobic autotrophs use inorganic molecules as their energy source and reduce O_2.

3.12 *Solvita® Maturity Index, n*—An index that incorporates two test parameters (NH_3 and CO_2) to yield color-coded test results. The test ranks compost on an index scale [1-8] of increasing compost maturity, i.e., a value of 8 indicates that the compost is resistant to further decomposition, biologically stable, and free of ammonia which can be toxic to plant growth.

3.13 *stability index, n*—the level of microbial activity in a sample of compost as determined by a respiration test; assumes a balanced nutrient status that favors microbial activity and the absence of toxins or other compounds that inhibit microbial respiration.

4. Summary of Test Methods

4.1 Although O_2 consumption and CO_2 evolution are related, the measurements are not always equivalent. Not all biological activity results in the complete mineralization of carbon to CO_2. Oxygen consumption rates may approximate aerobic biological activity more closely than CO_2 evolution rates.

4.2 Apparatus for CO_2 measurements are generally less expensive than those needed for measuring O_2. Additionally, CO_2 measurements are precise and simple, whereas O_2 consumption measurements are tedious and precision across replicates is difficult to maintain.

4.3 *Method 05.08-A Specific Oxygen Uptake Rate*—The rate of O_2 consumption is quantitatively measured using manometric and electrolytic respirometers, by measuring changes in O_2 concentrations with gas

chromatography or O_2 electrodes (Zibilske, 1994). The method for measuring changes in headspace O_2 concentrations with an O_2 electrode is described in this section.

4.3.1 The relative O_2 concentration in the head space of a closed flask containing a moist compost sample of known volume and mass, at known temperature and barometric pressure is monitored. The O_2 consumption rate per day is determined and reported relative to the total solids and organic matter contents of the material tested.

4.3.2 Details of the method are given by Ianotti et al., (1993 and 1994). Modifications of the published method (Department of Soil, Water, and Climate - Research Analytical Laboratory, University of Minnesota, St. Paul) are included to compensate for different types and conditions of MSW composts analyzed by that laboratory. All modifications and deviations from the published method are noted.

4.4 *Method 05.08-B Carbon Dioxide Evolution Rate*—The amount of CO_2 released biologically from a compost sample as a result of standardized incubation is reported per unit of volume or weight. This test is used to estimate the relative stability (biological activity) and maturity index of compost.

4.4.1 CO_2 evolution is an index of biological activity. Rates are measured in the headspace gas of static or dynamic systems with gas chromatography, infrared spectroscopy and alkali trapping and analysis, (e.g., manometric, titrimetric, conductimetric, or infrared spectrometric; Zibilske, 1994). The method for measuring CO_2 evolution rates in static chambers with alkali trapping and titration is described in this section.

4.4.2 Microorganisms utilize O_2 and generate CO_2 and water vapor during aerobic decomposition of organic matter. Microorganisms respire at high rates in biologically unstable compost and consume more O_2 and generate more CO_2 and water vapor than in more stable composts.

4.4.3 During anaerobic decomposition of feedstock materials, CO_2 and methane, CH_4, are generated.

4.5 *Method 05.08-C In-Situ Oxygen Refresh Rate*—Managed compost piles are turned or aerated to replenish O_2 that is consumed by microorganisms during decomposition of organic matter. Immediately after turning, or other aeration activity, one or more O_2 probes are inserted into the pile at various depths, and O_2 concentration is recorded at 10 minute intervals until the values stabilize. Results are expressed as percentage O_2 per interval of time.

4.6 *Method 05.08-D Dewar Self-Heating Test*—A standardized procedure used to measure self-heating as an indicator of biological activity. The difference in the maximum temperature produced by a sample of compost incubated for 10 days in a special, calibrated vessel relative to ambient temperature is measured.

4.7 *Method 05.08-E Solvita® Maturity Index*—A semi-quantitative (scaling) procedure used to determine carbon-dioxide (CO_2) and ammonia (NH_3) release into the closed headspace above a volumetric compost sample. The test provides a rapid and accurate determination of compost maturity.

4.7.1 Two determinations are performed simultaneously on one sample during a 4-h test period. The relative concentrations of evolved CO_2 and NH_3 are expressed on two corresponding color-indicator paddles. The color-coded paddles are pre-calibrated for a range of CO_2 evolution rates from approximately 2 through 30 mg CO_2-C per g OM per day, and a range of NH_3 concentrations equivalent to 200 through 20,000 mg of NH_3-N + NH_4-N per kg of compost (dw basis).

4.7.2 Color changes occur during the 4-h test period and express the relative concentrations of CO_2 and NH_3 in the compost sample. The *Solvita® Maturity Index* is derived from results of both tests and normally increases as both the CO_2 rate and NH_3 levels decline.

4.8 *Method 05.08-F Biologically Available Carbon*—A compost sample is prepared by optimizing moisture, pH, porosity, nutrients and temperature. The prepared sample is incubated for three days to reestablish a microbial community. One measure of CO_2-C is made in the headspace of the sample vessel to determine BAC-Respiration.

5. Significance and Use

5.1 *Methods 05.08-A and 05.08-B*—Respirometry is the measurement of CO_2 evolved or O_2 consumed by heterotrophic microorganisms within the compost and provides an estimate of biological activity of a composted material. Oxygen consumption during composting is influenced primarily by the rate of aerobic biological activity. Since aerobic activity is a function of compost stability, respiration rates are also related to compost stability.

5.1.1 Microorganisms utilize O_2 and generate CO_2 and water vapor during aerobic decomposition of organic matter. Microorganisms respire at high rates in biologically unstable compost and consume more O_2 and generate more CO_2 and water vapor than in more stable composts.

5.1.2 During anaerobic decomposition of feedstock materials, CO_2 and CH_4, methane, are generated.

5.2 *Method 05.08-C In-Situ Oxygen Refresh Rate*—This test is used to monitor or evaluate the relative aerobic status of compost under field conditions.

5.3 *Method 05.08-D Dewar Self-Heating Test*—The test was first introduced in Europe in 1982 by Jourdan and recently re-evaluated. Numerous workers have reported investigations on compost maturity and the heating traits of composts. The Dewar self-heating method was adopted as an official standard for stability by the German Department of the Environment in 1984 as a follow up to the 1982 Sewage Sludge Order.

5.3.1 The self-heating test based on Dewar flask measurement has merit as a general technique to evaluate compost stability and maturity, provided the general conditions of the test and the specific equipment are applied. The method may be utilized by producers under field conditions where a relatively stable room temperature of 20-25°C (but no more than 25°C) can be maintained around the vessel. In the laboratory, the Dewar method aids researchers in understanding the differences in idealized laboratory technique versus field observations. Teachers and environmental monitoring programs have found the test useful to demonstrate principles of compost aging. Because the results are expressed as temperature, they are easily understood and accepted by users and consumers.

5.3.2 The Dewar method is simple to use, applicable to the majority of composts produced, and only requires a small, standardized vessel and min/max thermometer.

5.3.3 The Dewar test integrates a number of factors present in normal composts and can reflect well with field observations about the stability status of compost. It does not provide the same type of data as the more precise laboratory respirometry procedures, but, like all respiration methods, (Dewar self-heating, CO_2-evolution, O_2 consumption), it gives a relative indication of the biological activity status of the compost as it pertains to biological stability..

5.4 *Method 05.08-E Solvita® Maturity Index*—An indexing system devised to rank compost maturity by indirectly measuring biological activity, or respiration, and chemical stability. The test is used to evaluate unknown compost products to help verify marketing claims for compost product shipments.

5.5 *Method 05.08-F Biologically Available Carbon*—Optimization of all edaphic parameters, except carbon, promotes the highest possible respiration rate, where carbon remains the only limiting factor. This test determines whether the compost respiration process will reactivate after compost is introduced to agricultural conditions.

6. Interference and Limitations

6.1 Many adiabatic factors affect the activity of microorganisms in composts and must be carefully maintained to obtain precise and accurate readings for stability. Respirometry tests require a balanced nutrient status that favors microbial activity and the absence of toxins or other compounds that inhibit microbial respiration.

6.1.1 Microbiological respiration depends on moisture relative to the WHC, rather than to moisture content based upon sample wet-weight.

6.1.2 Generally, test compost samples that have a moisture content below 35%, wet weight basis, will be biologically dormant and a respiration rate determination will be artificially low. Samples must be moistened to 70% to 85% of WHC (typically 45% to 50%, wet weight basis), and allowed to equilibrate for at least 24 h at a specified temperature as per the method, prior to analysis. Incubation for up to three days may be necessary with some compost samples.

6.1.3 Samples removed from high temperature zones in compost piles (55-65°C) harbor thermophilic microorganisms that may not be active at low, mesophilic temperatures (below 37°C). Compost samples must contain subsamples from various temperature zones of a pile and be tested at a temperature of 34°C to avoid temperature pitfalls. Iannotti et al. (1993, 1994) provides more details on this potential interference.

COMMENT—If the compost is still at high temperatures, then it may be a waste of money to test for stability if the goal is testing for compost quality. This may be important if testing for other purposes, i.e., tracking metabolic activity during the course of compost production.

NOTE 1—Testing compost for stability at 37°C may be selectively testing for organisms in the upper range of the mesophilic organisms and may not be indicative of what can happen in the soil after the compost is incorporated. It is very possible to get a misleading analysis of compost stability when testing at 37°C. It is suggested by leading compost stability researchers that pre-incubation at 25-28°C and testing at 34°C would be more reflective of the actual compost metabolic activity potential.

6.1.4 Samples that are over-moist, tightly packed in a sealed container and shipped at temperatures above 4°C, usually arrive in an anaerobic state that is unrepresentative of the sample source and are not suitable for analysis.

6.1.5 Toxic compounds and some heavy metals that occasionally contaminate compost can inhibit microbial respiration.

6.2 *Method 05.08-A Specific Oxygen Uptake Rate*—Saprophytic fungi may heavily colonize properly moistened samples. This condition is usually

associated with very high levels of NH_4 (> 500 mg kg^{-1}) and unstable material. Fungal mycelium serve as a food source for bacteria and will induce a flush of bacterial activity during incubation and upon aeration. If the presence of these fungi is not diminished through incubation prior to respirometry measurements, respiration measures will indicate high O_2 uptake rates. If this condition persists, the presence of mycelium must be noted in the laboratory report.

6.2.1 In addition to the other issues for Method 05.08-A, the presence of inert material in the sub-sample used to determine sample organic matter must be evaluated. It is recommended that an inert content estimate be made on a parallel sub-sample and that organic matter content (biological volatile solids) be estimated using a clean sample, free from inerts. Refer to *Method 02.02-C Man Made Inert Removal and Classification* for a detailed description of this step in sample preparation.

6.3 *Method 05.08-B Carbon Dioxide Evolution Rate:*

6.3.1 Depletion of O_2 in the headspace of incubation vessels may result in decreased biological activity. This can be avoided with 4-L containers and approximately 25-g samples, except for very unstable composts.

6.3.2 Alkali trapping of CO_2 in the headspace of the incubation container may reduce the partial pressure of CO_2 enough to upset the carbonate equilibrium in the compost sample. For this reason, some of the measured CO_2 may be derived from inorganic sources such as carbonates.

6.4 *Method 05.08-C In-Situ Oxygen Refresh Rate*—Frequent equipment re-calibration and cleaning is imperative to attain reliable readings. Standard gases of fixed O_2 content may be blended to achieve 1, 5, 10 and 15% O_2 for exact calibration trials. Apparatus not adapted with temperature compensation can provide unpredictable results.

6.5 *Method 05.08-D Dewar Self-Heating Test*—A compost sample passed through a 20-mm sieve improves test precision, but results in slightly higher temperatures.

6.5.1 Optimal compost sample moisture conditions must be maintained for successful application of the Dewar self-heating procedure. As with other respiration tests, if sample moisture is too low (~30%), or too high (~65%), the Dewar class maturity determination will result in false positive. Originally, the European procedure called for optimizing moisture by partial pre-drying and remoistening to a set point of ~30% moisture. This moisture level is too low for compost with a high WHC and correspondingly low bulk density.

6.5.2 The optimal moisture to conduct a Dewar test often depends on porosity of the material. The less porous the compost material, the more air transfer and heating are limited. Since moisture absorbs heat, it is understandable that the lowest optimal amount of water will produce the highest heating in the Dewar test. With experience, specific users will evolve appropriate methods that give reliable results.

6.6 *Method 05.08-E Solvita® Maturity Index:*

6.6.1 *Volatile Fatty Acids*—High levels of volatile fatty acids (VFA) may interfere positively with Solvita® and negatively with other respiration tests. Compost samples that produce a *Solvita®* #1 [bright yellow] commonly contain high levels of VFA. The maximum interference observed for VFA-containing samples is approximately one color change for Solvita®, but must be separately determined for other respiration procedures.

6.6.2 *Immature Composts*—High levels of ammonia (NH_3) in compost may lower the CO_2-evolution rate (interfere negatively) as indicated by Solvita®. This interference is factored out by the ammonia-test result of the second gel-paddle.

6.6.3 *Active Denitrification*—In certain cases of composts that are anaerobic or undergoing active denitrification, nitrous-oxide can be produced resulting in an off-coloring of the Solvita® gel. Such samples invariably give high test results for nitrite, a phytotoxic intermediate of nitrification or denitrification.

6.6.4 *Temperature*—The Solvita® test is normally run at room-temperature for 4 hours. If the test is run at temperatures outside this range (20-25°C), results should be read at more or less than four hours. Compost samples collected from active piles must be re-equilibrated at room temperature before testing is started.

6.6.5 *Shelf-life*—The Solvita® ge1-pack can be stored for approximately one year. Shelf-life can be extended with refrigerated storage (4ºC).

7. Sample Handling

7.1 *Methods 05.08-A and 05.08-B*—See methods for pre-incubation requirements.

7.1.1 *Sample Moisture Status*—The moisture level should be judged by the squeeze test at sampling. If the compost is too wet or too dry, it is advisable to postpone sample collection and to adjust pile moisture. Changes made to a sample after collection may unpredictably bias the test result.

7.1.1.1 To ensure test results that more accurately represent compost material stability, the sample must be in a condition where aerobic microbes flourish. If samples are too wet (potentially anaerobic) or too dry, they need to be brought to the proper moisture content (70% to 85% of WHC). For most samples this is between 40-50% moisture. Samples with a high bulk density, approximately 0.75 g cm^3, and low organic matter are usually over-saturated at 40-50% moisture and require less water (30-40% moisture, wet weight basis). Conversely, samples with a low bulk density and a very high WHC may be too dry with only 40-50% moisture.

7.1.1.2 *Excessively Dry Samples*—Spread a 600 cm^3 sample aliquot uniformly onto a clean plastic lab tray (18 × 22 in.). Sprinkle with deionized water while thoroughly mixing by hand until moisture content of 70% to 85% of WHC is attained. Use caution to maintain a loose texture and avoid aggregating the compost test aliquot into clumps or balls. Unwanted aggregates form most easily when handling material that is too wet. Transfer two 300 cm^{-3} aliquots of moistened material to two 1 quart vegetable Ziploc® brand plastic vegetable bags. Place the bags in a high humidity incubator set at 34°C overnight to continue moisture equilibration.

7.1.1.3 *Excessively Wet Samples*—A sample is too wet if water can be squeezed from a fist-full of material. This precautionary observation should be performed when the sample is received at the laboratory, before sample splitting, sieving or initial sample preparation. If too wet, spread a 600 cm^3 sample aliquot uniformly on a tray and allow to dry until no free water is evident. If the sample is left in open air for extended periods (over night), the sample may become excessively dry and will require re-moistening. To prevent this problem, a perforated sheet of aluminum foil, paper or plastic may be used to cover the tray containing the excessively wet sample.

7.2 *Method 05.08-C In-Situ Oxygen Refresh Rate*—This test is initiated immediately after turning (windrow systems) or during a complete aeration cycle (closed, static systems).

7.3 *Method 05.08-D Dewar Self-Heating Test*—A well blended representative compost sample with a moisture content of 70-85% of WHC is cooled to ambient temperature (18-22°C). Sieving compost through a 20-mm sieve improves test precision, but results in slightly higher temperatures.

7.4 *Method 05.08-E Solvita® Maturity Index:*

7.4.1 *Composite Sample*—A well-blended composite sample representing the average of the whole pile to be tested (or any specified portion thereof) is gathered by collecting several sub-samples throughout the pile with a shovel or other sampling device. Homogeneous samples are most easily collected immediately after turning a pile. Large fragments such wood chips and other bulking agents (> ½ in.) are too large for the *Solvita®* jar and should be removed or screened from the compost sample before testing.

7.4.2 *Sample Temperature*—Hot (thermophilic) samples must be cooled to room temperature before testing.

7.4.3 *Sample Moisture Content*—Optimal moisture is absolutely necessary. The moisture level is judged by the squeeze test at sampling. If the compost is too wet or too dry, it is advisable to postpone sample collection for the *Solvita®* test and to adjust pile moisture. Changes made to a sample after collection may unpredictably bias the *Solvita®* test result.

NOTE 3—A squeeze test is performed with a handful of compost. A moist sample will clump when tightly squeezed. A sample with optimal moisture will feel wet, but not produce free water. A sample that is too dry is dusty and will not clump with hard squeezing.

7.5 *Method 05.08-F Biologically Available Carbon*—Store at 4°C for no more than three days until tested. Samples should be prepared for analysis upon receipt. See method.

Test Method: Respirometry: SOUR: Specific Oxygen Uptake Rate						**Units:** mg O_2 g^{-1} (TS, OM) d^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.08-A		05.08-A		05.08-A		05.08-A

05.08-A SOUR: SPECIFIC OXYGEN UPTAKE RATE

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

COMMENT—Automated systems used in place of the apparatus described in this section provide significantly lower outcomes for moderate to highly active compost samples, (e.g., Columbus Instruments describes a system, MicroOxymax, at http://www.colinst.com, 950 North Hague Avenue; Columbus, OH 43204-2121 USA).

8. Apparatus for Method A

8.1 *Oxygen meter*—(e.g., YSI oxygen meter, digital model no. 58, or equal).

8.2 *Oxygen sensor*—(e.g., YSI oxygen probes, model no. 5718, or equal).

8.3 *Data logger*—(e.g., A/D conversion board for PC computer, No. G-08109-25; software, No. G-08109-32. Cole-Parmer Instruments, or equal).

8.4 *Computer*—IBM PC-XT, minimum.

8.5 *Incubator*—for setting at 34°C, with humidity control system.

8.6 *Water bath*—set at 34°C, with cover.

8.7 *Vaporizer-humidifier*—cool, (e.g., Hankscraft No. 240), if incubator is not equipped with humidity control system.

8.8 *Erlenmeyer flask*—1-L, fitted with a two-hole stopper and one short glass delivery tube, and a fritted sparger tube.

8.9 *Erlenmeyer flasks*—500-mL (one per sample).

8.10 *Evaporation dish*—borosilicate glass beakers, 100-mL and 250-mL (per sample).

8.11 *Pinch clamps*—for 0.94 cm ($^3/_8$ in.) o.d. flexible plastic tubing.

8.12 *Tubing*—0.94 cm ($^3/_8$ in.) o.d. flexible rubber or plastic.

8.13 *T-Connectors*—plastic T-fittings for 0.94 cm ($^3/_8$ in.) o.d. flexible rubber or plastic.

8.14 *Stoppers*—rubber, No. 5.

8.15 *Pipettes*—Pasteur, 15 cm (6 in.).

8.16 *Mesh*—firm nylon, 1 to 2 mm; cut in approximately 6.25 cm (2.5 in.) diameter disc to fit on inside base of a 500-mL Erlenmeyer flask.

8.17 *Vinyl tubing*—0.94 cm (⅜ in.) flexible tubing shaped as ring and attached to mesh disc.

8.18 *Cotton cloth*—45 cm × 60 cm tea towel or equal.

8.19 *Beakers*—200-mL.

8.20 *Hypodermic needle*—1-cc tuberculin syringe, 25 × ⅝ in., (e.g., Pharmasela. Catalog No. 7021D, or equivalent).

8.21 *Check valve*—for water bath aeration tubing.

8.22 *Marking pen*—for glass.

8.23 *Weight rings*—to anchor Erlenmeyer flasks in water bath.

8.24 *Drying oven*—forced-air, vented, set at 70±5°C.

8.25 *Muffle furnace*—vented, set at 550°C.

8.26 *Beakers*—100-mL, borosilicate glass.

8.27 *Analytical balance*—accurate to ± 0.001 g.

9. Reagents and Materials for Method A

9.1 *Water*—deionized, 17 MΩ·cm minimum resistivity.

9.2 *Glycerol.*

9.3 *Tween® 20*—polyoxyephene (20) sorbitan monolaurate, (available through JT Baker Chemical Co, NJ, and others).

9.4 *Bags*—plastic, with vents or perforations, 0.25 L (1 qt) size, (e.g., Ziploc® brand vegetable bags with freshness vents).

10. Apparatus Assembly and Sample Preparation for Method A

10.1 *Sample Cleaning*—remove all large pieces of plastic (>4.0-mm) and other non-biodegradable combustibles from the compost sample.

10.2 *Pre-Incubation*—after adjusting sample moisture, transfer 300 cm^3 aliquots into two bags and close the bags, (e.g., one qt size Ziploc® plastic vegetable bags). Place the bags on a lower shelf of an

incubator set at 34°C and cover loosely (as a blanket) with a wet cotton cloth (wring out after soaking in deionized water). The wet cotton tea towel minimizes evaporative water loss near the vents in the vegetable bag.

10.2.1.1 *Incubator Humidity*—Set humidity at approximately 99% relative humidity. If incubator is not equipped with humidity control system, place a humidifier on a laboratory tray on the top shelf of the incubator. Plug the humidifier into the 24 h timer set to repeat periods of 3 h *ON* and 1 h *OFF*.

NOTE 1A—Use of vented bags, wet covering cloth and humidifier is an addition to the method as described by Iannotti, et al.

10.2.2 *Pre-Incubation*—Incubate samples for approximately 24 h, pending condition of the samples. During the incubation period check the samples daily for signs of anaerobic conditions.

10.2.2.1 If problems are observed, carefully mix the sample by gently shaking or stirring. This action assists aeration by breaking and blending anaerobic pockets throughout the sample. The presence of white fungal mycelium is usually associated with high NH_4 and unstable material.

CAUTION—If samples are too moist, any handling of the sample can result in the formation of clumps and balls. If this condition develops, prepare a new sample and reinitiate the incubation process.

10.3 *Flask Preparation*—before adding a compost aliquot to the flask, assemble a mesh support that will promote free movement of air below and through the sample. Refer to Fig 05.08-A1 for illustration.

10.3.1 Form a ~5-cm (2-in.) i.d. ring with flexible 0.94 cm (⅜ in.) tubing.

10.3.2 Cut a disc of nylon mesh to fit inside bottom of 500-mL Erlenmeyer flask

10.3.3 Attach the flexible ring and disc of mesh using nylon string.

10.3.4 Push the assembled disc with tubing into the mouth of flask and force it to fit flat on the flask bottom with the ring of tubing facing down.

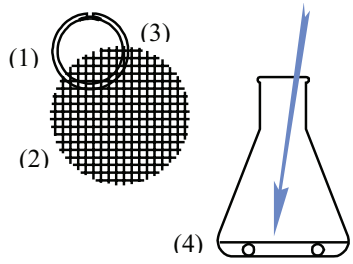


Fig 05.08-A1 Flask preparation.

10.3.5 Weigh and record the mass of the flask fitted with nylon mesh and tubing ring.

10.4 *Flask Stopper Assemblies*—Two separate stoppers are used during this test, during aeration, and during O_2 measurement. Refer to Fig 05.08-A2 for illustration.

10.4.1 Bore two 7.5-mm adjacent holes into the first No. 5 rubber stopper and insert a pasteur pipette and straight glass tubing into the holes.

10.4.2 Attach a 20-cm (8-in.) section of flexible tubing fitted with check valve onto the straight glass tubing.

10.4.3 Connect the pipette and air-feed from the fritted glass sparger tube into the 1-L flask (not illustrated).

10.4.4 Bore one 1.8-cm hole into the second No. 5 rubber stopper and insert the O_2 sensor.

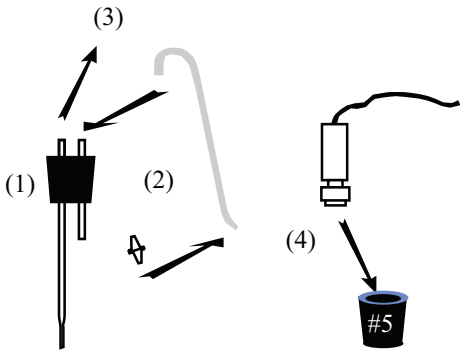


Fig 05.08-A2 Flask stopper assemblies.

10.5 Gently transfer 250 cm^3 aliquot of pre-incubated and properly moistened compost into the flask through a funnel with a 200-mL beaker. Refer to Fig 05.08-A3 for illustration.

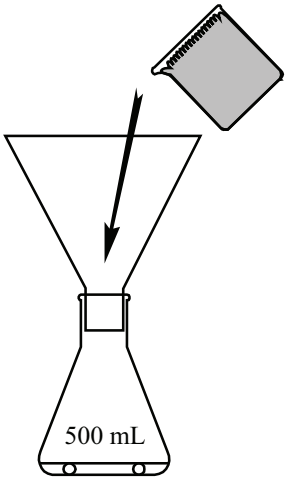


Fig 05.08-A3 Transferring compost aliquot to incubation flask.

10.5.1 Weigh and record the mass of the sample-filled flask.

NOTE 2A—The published method calls for a 60 g dw equivalent sample of moist compost. This method calls for 250 cm^3 of pre-incubated and properly moistened compost. Use of the volume measure improves experimental precision of the respiration test. This modification improves segregation of samples with similar solids content, but dissimilar physical characteristics, i.e., different bulk densities.

10.6 *Test Aliquot Moisture*—Transfer the remaining incubated compost material (approximately 50 cm^3) into tared 100-mL beaker, record the gross weight.

10.6.1 Oven dry at 70±5°C until weigh change diminishes to nil. Record the oven dry weight for determination of sample total solids content (TS, wet basis) as described in Method 03.09 Total Solids and Moisture.

10.6.2 Ash the oven-dried aliquot at 550°C for 2 h as described in Method 05.07 LOI Organic Matter.

10.7 *Equilibrate Temperature and Aerate Sample*—Refer to Fig 05.08-A4 for illustration.

10.7.1 Fill water bath with tap water to a depth of about 6 cm and pre-heat to 34°C.

10.7.2 Place flasks containing pre-incubated compost in the pre-heated water bath and anchor with weight rings.

10.7.3 Insert aeration assembly into mouth of flask.

10.7.4 Position tip of pipette between base of flask and support mesh.

10.7.5 Place flask fitted with weight ring into 34°C water bath.

10.7.6 Attach tubing from sparger to inlet end of pipette.

10.7.7 Adjust aeration rate to allow approximately one bubble per s to pass from outlet positioned below water line.

10.7.8 Aerate using sparged air for one h to equilibrate sample temperature.

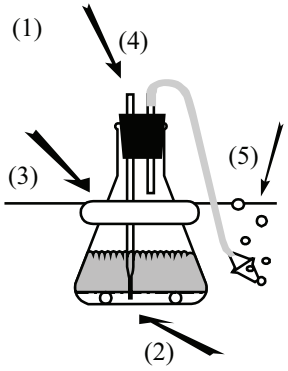


Fig 05.08-A4 Equilibrate temperature and aerate sample.

10.8 *Equilibrate Flask Air Pressure:*

10.8.1 Insert O_2 sensor assembly into the flasks without removing them from the water bath. To minimize air leakage use glycerol, to seal the O_2 sensor into the stopper and to seal the stopper into the flask.

10.8.2 Equilibrate the air pressure within flask to that outside the flask. Insert a hypodermic needle through the stopper fitted with the O_2 sensor before placing the stopper into the flask. Remove the needle after the stopper is securely in place and pressure within the flask equilibrates to that outside of the flask, approximately five min.

10.8.3 Check electronic cable connection from O_2 sensor assembly to computer.

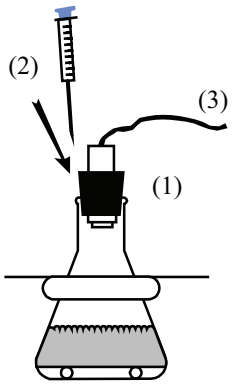


Fig 05.08-A5 Equilibrate barometric pressure inside flask.

NOTE 3A—The hypodermic needle relieves pressure due to displacement that occurs while inserting the stopper into the flask. The needle allows the air pressure inside the flask to equilibrate with that outside the flask, i.e., a parameter representing atmospheric pressure in equation 13.1. The pin-hole created by the needle seals itself. Monitor the texture of the stopper with continued use. If the stopper becomes slightly brittle, leaks will occur.

10.9 *Water Blank*—Run a parallel deionized H_2O blank to detect systematic errors. Transfer 250 mL deionized H_2O into a clean flask, treating it as a compost sample to measure respiration.

NOTE 4A—The original method does not call for a blank. The blank reveals non-linear changes in measured O_2 percent that may be attributed to systematic error, (e.g., variations in water bath temperature, voltage fluctuation and other factors not attributable to compost stability).

11. Procedures for Method A

11.1 *Apparatus*—Assemble apparatus as described in step 10.

11.2 *Pre-Incubation*—Perform all incubation and equilibration steps as described in step 10, above.

11.3 *Record Headspace Oxygen Concentration Change Over Time*—Set the data logger to record O_2 uptake (%) at 1 min intervals. Record changes in

percent O_2 within each flask for at least 90 min. Avoid long runs (>10 h) that promote anaerobic conditions in the flask. Anaerobic conditions will damage the O_2 sensors.

NOTE 6A—The original method calls for longer measurement intervals (10 min) over a shorter experiment duration (1 h). More consistent results and improved ease in diagnosing systematic errors are possible when measurements are recorded at short intervals (1 min) over a longer period of time (1.5 h).

11.4 *Determine Volume of Air in Flask (mL)*—Mark the neck of the flask at a point corresponding to the bottom or base of the O_2 probe assembly. Remove the probe assembly and flask from the water bath. Partially fill the flask (~80%) with deionized H_2O.

NOTE 7A—Avoid formation of bubbles and foam. Partially fill the flask with water to saturate sample. Allow the sample to rest for approximately 2 h; gently stir using a nylon or glass stirring rod; then fill to volume with water, i.e., to the mark with water. Weigh and record the gross weight of the flask, water and compost sample. Add two drops of Tween® 20 to diminish the formation of bubbles and to increase absorption. Calculate the net weight of water by subtracting the flask tare weight and the calculated oven dry weight of the compost sample from the gross weight. Assume 1 g of H_2O is equivalent to 1 mL of air.

11.5 *Data Analysis and Calculations*—Upon completion of the 90 min run, transfer the logged O_2 uptake data to a spreadsheet and create a scatter chart of O_2% versus time (t, min). Select a linear segment on the chart (at least 30 min from data set) to calculate the slope for O_2 uptake. Usually, the rate becomes relatively linear ($r > 0.99$) after the 20-30 min mark. The slope of deionized water blanks should be between -0.01 and -0.02 ($\Delta[O_2]\ \Delta t^{-1}$).

12. Trouble Shooting for Method A

12.1 *Aeration*—Sample aeration during temperature equilibration must be uniform across all samples (flasks) in water bath. Periodically check bubble rate (1 bubble per s). Correct any deviations by either tightening or loosening clamps on air delivery tubes.

12.2 *Temperature and Pressure Fluctuations*—Increasing temperature and pressure accelerate the rate of O_2 uptake and decreasing temperature and pressure decelerates uptake rates.

13. Calculations and Corrections for Method A

13.1 *Determine Specific Oxygen Uptake Rate, mg O_2 g^{-1} (TS, OM) d^{-1}:*

$$SOUR = 0.196 \times [V \div 1000] \times P \times [MW \times 1000] \times S \times 1440 \div [R \times T \times X \times M]$$

Equation 13.1

13.2 *The simplified form of the SOUR formula combining all constants, mg O_2 g^{-1} (TS, OM) d^{-1}:*

$$SOUR = [(1.1 \times 10^5) \times V \times P \times S] \div [T \times X \times M]$$

Equation 13.2

where:

SOUR = specific oxygen uptake rate, mg O_2 g^{-1} (TS, OM) d^{-1}
V = volume of air in flask, mL,
P = atmospheric pressure at elevation of measurement, atm,
MW = molecular weight of O_2 = 32 g mol^{-1},
R = ideal gas constant, 0.08206 L atm mol^{-1} K^{-1},
T = temperature in degrees, °K (°C+273)
S = slope of change in percent O_2 saturation per minute divided by 100, (e.g., the change from 100% to 90% in one minute, S = 0.1 min^{-1}),
X = wet weight of compost test aliquot, g
M = mass unit, fraction of total solids (TS) and organic matter (OM) of a parallel sample aliquot, i.e., 0.00-1.00, g g^{-1} wet basis, at 70±5°C, and 550°C dw basis.
1440 = conversion of minutes to days,
1000 = conversion of mL to L and mg to g, unitless, and
0.196 = the fraction of O_2 in saturated air at 34°C.

NOTE 8A—The formula presented above was re-derived by F. Michel and modified from that originally published by Iannotti, et.al., to allow accurate determines of specific O_2 uptake rates at any location or elevation.

Test Method: Respirometry. Carbon Dioxide Evolution Rate						**Units:** mg CO_2-C g^{-1} (TS, OM) d^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.08-B		05.08-B		05.08-B		05.08-B

05.08-B CARBON DIOXIDE EVOLUTION RATE

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

COMMENT—Automated systems used in place of the apparatus described in this section provide significantly lower outcomes for moderate to highly active compost samples, (e.g., Columbus Instruments describes a system, MicroOxymax, at http://www.colinst.com, 950 North Hague Avenue; Columbus, OH 43204-2121 USA).

14. Apparatus for Method B

14.1 *Incubator*—capable of sustaining constant temperature of 25-28°C with near-100% relative humidity.

14.2 *Beakers*—50-mL, glass.

14.3 *Incubation Vessel*—200-mL Erlenmeyer flask.

14.4 *Respiration Flask*—4-L Containers, mason jars or equivalent that can be readily sealed.

14.5 *Magnetic Stirrer.*

14.6 *Titration Burette.*

14.7 *Dispensing Pipettes.*

14.8 *Flask*—volumetric, 1-L.

15. Reagents and Materials for Method B

15.1 *Sodium Hydroxide (1M)*—Place 40 g of NaOH pellets into a 1-L volumetric flask. Add approximately 500 mL deionized water. Dissolve completely, cool, and add deionized water to bring to 1 L and stopper tightly.

15.2 *Barium chloride (~0.5N)*—Place 120 g of $BaCl_2 \cdot 2H_2O$ into 1 L of CO_2 free distilled water. Mix well with a magnetic stirrer until dissolved and stopper tightly.

15.3 *Phenolphthalein Indicator Solution*—Dissolve 5 g solid phenolphthalein in 500 mL 95% ethyl or isopropyl alcohol, and add 500 mL distilled water. Mix well with a magnetic stirrer. If necessary, add 0.02*N* NaOH dropwise until a faint pink color appears in solution.

15.4 *Hydrochloric Acid (0.5M)*—Make 6*M* HCl, by placing 500 mL distilled water into a 1-L volumetric flask and slowly add 500 mL concentrated HCl. Make 0.5*M* HCl, by placing 500 mL distilled water into a 1-L volumetric flask, adding 83 mL 6*N* HCl, and filling to volume mark with distilled water. Store in a glass carboy.

15.5 *Acid Normalization (mol L^{-1})*—Hydrochloric acid for respiration titrations should be standardized after preparation and on a monthly basis thereafter. Over time, the evaporation of water, absorption of CO_2 from the air and other factors may change the normality of the acid.

15.5.1 Weigh between 0.400 and 1.000 g of THAM (tris hydroxymethyl aminomethane) in a 50-mL beaker. Add approximately 20 mL of distilled water. Add two drops of bromocresol green/methyl red mixed indicator. Titrate with HCl to the endpoint denoted by a color change from green to red.

15.5.2 *Determine Normality of Acid*—Follow equation.

$$A = B \div [C \times D] \qquad \textit{Equation 15.5.2}$$

where:

A = normality of HCl, mol L^{-1},
B = 0.400 to 1.000 g of THAM (tris hydroxymethyl aminomethane), g,
C = molecular weight (gmw) THAM, 121.14 g mol^{-1}, and
D = volume of HCl, L.

15.5.3 Repeat the standardization procedure two times and average results. Record the normality and the date in the lab notebook and on the carboy.

16. Procedure for Method B

16.1 *Pre-Incubation*—Prepare approximately 30 g of as-received moist material as described.

16.1.1 *Sample Moisture Adjustment*—Use the squeeze test to approximate the moisture status of each sample. Optimal moisture of approximately 70% to 85% of WHC is absolutely required. It is important to clearly report sample moisture adjustment. The moisture adjustment step must be included as commentary in the data reporting process. This is most readily accomplished by reporting the sample moisture before and after adjustment.

NOTE 1C—A squeeze test is performed with a handful of compost. A moist sample will clump when tightly squeezed. A sample with optimal moisture will feel wet, but not produce free water. A sample that is too dry is dusty and will not clump with hard squeezing.

16.1.2 Allow the samples to set or pre-incubate at room temperature (25-28°C) for a specified period of time but not less than 24 h and not more than three days. Use care to minimize sample moisture loss by maintaining high humidity conditions in the incubator, or other large, closed container.

16.1.3 The purpose of a pre-incubation period is to allow microorganisms in the compost to adapt to the mesophilic environment in which the test is conducted.

16.2 *Determination of Total Solids and Organic Matter Content*—Determine the total solids and moisture content of the sample in preparation for this respirometry test. Refer to Method 03.09 Total Solids and Moisture. Determine the organic matter content of the parallel sample using Method 05.07-A LOI Organic matter.

16.3 *Incubation*—Transfer 25.0 g pre-incubated compost sample into the incubation vessel set at 34°C. Record the weight of this sample to the nearest 0.01 g.

NOTE 2C—A 30-mL NaOH trap should be used initially for unstable materials to insure all the NaOH is not neutralized.

16.3.1 Transfer 20 mL of 1*M* NaOH to a 50-mL beaker. Place the NaOH and the compost sample into an incubation vessel. Close the lid tightly and place it in an incubator set at 34°C. Report the incubation temperature selected if different than that called for in this protocol.

16.3.2 Set up a blank by placing a 20 mL aliquot of 1*M* NaOH into an incubation vessel without a compost sample.

16.3.3 Record the date and time the first sample was prepared.

16.4 *Titration*—The amount of CO_2 absorbed by each NaOH trap is determined daily over a four day period by back titration of the residual with normalized HCl according to the procedure outlined below.

16.4.1 Open the incubation vessel and remove the sample container and beaker containing NaOH.

16.4.1.1 *Optional Step*—Remove and weigh the beaker containing the compost sample. This step is included to track sample moisture through the 4-day experiment. Calculate sample moisture for each of the four titrations.

16.4.2 Transfer the NaOH to a 200-mL Erlenmeyer flask rinsing with distilled water and add approximately 20 mL of 0.5*N* $BaCl_2 \cdot 2H_2O$.

NOTE 3C—If a 30-mL NaOH trap is used, 40 mL $BaCl_2 \cdot H_2O$ should be added.

16.4.3 Add two to three drops of phenolphthalein indicator.

16.4.4 After zeroing the burette, add HCl until the solution begins to turn clear. Use a magnetic stirrer to mix the solution while adding the acid. The endpoint has been reached when addition of one drop of HCl turns the solution from pink to clear.

16.4.5 Record the date and time the first sample was titrated, the normality of the HCl used and the volume of HCl required to achieve the endpoint.

16.4.6 Place the sample back into the incubation vessel with a fresh amount of NaOH.

16.5 Perform calculations for each of the four titrations. Report the average CO_2 evolution rate on the basis of both totals solids and organic matter, as mg CO_2-C g^{-1} TS d^{-1} and mg CO_2-C g^{-1} OM d^{-1}.

17. Calculations for Method B

17.1 Calculate CO_2 Evolution for each titration:

$$A = [(B - C) \times (D \times E)] \div [F \times G] \qquad \textit{Equation 17.1}$$

where:

A = mg CO_2-C g^{-1} (TS, OM) d^{-1},
B = volume of standardized HCl used for blank titration, mL,
C = volume of standardized HCl used for sample titration, mL,
D = normality of standardized HCl, mol_c L^{-1},
E = 6 = equivalent weight of CO_2-C in NaOH,
F = moist weight of sample in container, g, and
G = mass unit, fraction of total solids (TS) and organic matter (OM) determined on a parallel sample, 0.00-1.00, g g^{-1} wet basis determined at 70±5°C or 550°C dw basis, respectively.

17.2 Calculate the average rate of CO_2 evolution:

$$H = \Sigma A \div I \qquad \textit{Equation 17.2}$$

where:

H = average mg CO_2-C g^{-1} (TS, OM) d^{-1},
ΣA = tally CO_2 evolution measures from days one through four, from Equation 17.1, and
I = duration of experiment, four d.

Test Method: Respirometry. In-Situ Oxygen Refresh Rate						**Units:** % O_2 hr^{-1} chart		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.08-C		05.08-C		05.08-C		05.08-C

05.08-C IN-SITU OXYGEN REFRESH RATE

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

SUBMITTED BY William F. Brinton, Woods End Research.

18. Apparatus for Method C

18.1 *Oxygen probe*—configuration adapted to field data collection, or equivalent manufactured device

18.1.1 *Oxygen sensor*—capable of ±0.5% O_2 readings, with temperature compensator.

18.1.2 *Probe*—galvanized steel pipe, 0.3-cm (⅛ in.) I.D. and ~1.3-m length.

18.1.3 *Filter*—in-line, for water vapor.

18.1.4 *Squeeze bulb*—with a per squeeze volume of five mL, to extract air.

18.2 *Data logger (optional)*—digital data logger, minimum specification to log at a ten-minute interval for two h.

19. Procedures for Method C

19.1 Pre-calibrate the O_2 probe to ambient at 20.9% O_2.

19.2 Perform aeration procedure, (e.g., turn windrow, cycle fans, etc., pending composting system).

19.3 Insert probe to a specified depth. The probe remains in position until the end of reading the refresh test.

NOTE 1C—It is advisable to repeat this test at various pile positions and depths to test for uniformity of pile aeration characteristics.

19.3.1 *Sampling Strategies*—stratify the compost pile or bin into spatial zones, where each zone represents a relative position within the compost, (e.g., core of the bulk and margins at various depths). Perform the test at all positions to evaluate aeration uniformity.

19.4 Record percent O_2 at a ten min interval for two h or until readings level off. Round the O_2 reading to the nearest whole unit.

19.5 Graph results with Y-axis as O_2 percent and X-axis as time in minutes.

20. Interpretation of Results for Method C

20.1 Refresh rate is considered excellent if pile O_2 does not fall below 5% within two h. If pile O_2 falls under 2% in thirty min, then odor events are likely. The interpretation must consider the feedstock types, (e.g., a higher minimum O_2 reading may be appropriate for feedstocks that are predisposed to produce odor).

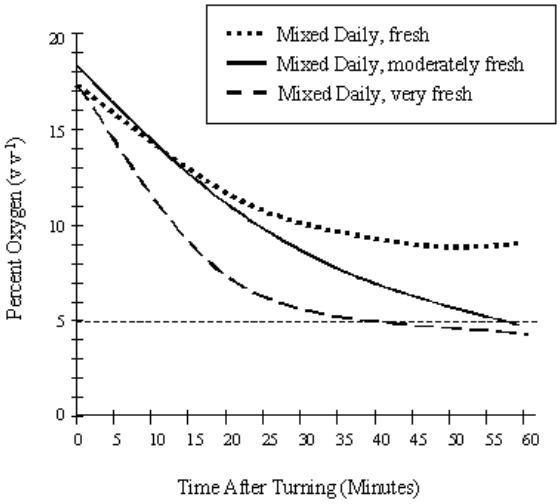


Fig 05.08-C1 Idealized oxygen refresh rate test results from monitoring three piles of varying condition during active phases of the composting process.

Test Method: Respirometry. Dewar Self-Heating Test							**Units:** Δ°C	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.08-D		05.08-D				05.08-D

05.08-D DEWAR SELF-HEATING TEST

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

ADAPTED FROM—Woods End Research Laboratory, Inc. in Mt. Vernon, ME. Dr. William Brinton, President of Woods End Research Laboratory; Dr. Mary Droffner, Director of Microbiology; Eric Evans, Laboratory Director; and Richard Brinton, director of Woods End UK, Stroud, England office. A Dewar Self-heating Kit and set of instructions are available by writing to Woods End at the following address: Woods End Research Laboratory, Inc.; P.O. Box 197 Mt. Vernon, ME 04352,. Tel: 207.293.2457. The Dewar test was first formulated in Stuttgart Germany by Jourdan (1982).

21. Apparatus for Method D

NOTE 1D—The Dewar kit presented here consists of three parts, each replaceable separately. The proper materials may be readily obtained from major scientific supply houses.

21.1 *Dewar vessel*—2-L, 100 mm i.d., steel-encased,

NOTE 2D—The inner diameter and volume specifications of the Dewar vessel must be correct.

21.2 *Thermometer*—dual scale min-max inside/outside digital thermometer with ±1°C increments over a range of 10°C through 80°C, and

21.3 *Thermocouple probe*—30 cm, attached to a PVC wand for insertion into vessel.

22. Reagents and Materials for Method D

22.1 *None required.*

23. Procedure for Method D

23.1 Separate approximately 2 L of the representative compost sample.

23.2 Determine sample moisture.

23.2.1 Add or remove moisture if the sample is too dry or wet.

23.3 Equilibrate compost sample to ambient temperature (18-22°C).

23.4 Fill the Dewar flask with sample material (~2 L). Gently shake the filled flask to simulate natural settling.

23.5 Insert the high-point reading thermocouple probe into the flask to a point about 5 cm (2 in.) from the bottom of the flask.

NOTE 3D—Do not push against the bottom of the flask.

23.5.1 The thermometer records both maximum ambient and sample temperatures.

23.5.2 Maintain ambient temperature and vessel at 18°C - 22°C for the duration of the test.

23.6 Record ambient and sample temperatures on a daily basis, and days of readings.

23.6.1 Compost will normally achieve its highest temperature within three to five days. If the compost sample has been exposed to very cold conditions or requires remoistening, maximum temperature may not be achieved until days five to ten.

23.6.2 Continue recording temperatures for at least two days after maximum temperature is reached.

24. Calculations for Method D

24.1 *Net Temperature Rise:*

$$R = H - A \qquad \textit{Equation 24.1}$$

where:

R = net temperature rise, Δ°C,
H = highest temperature recorded over test period, °C, and
A = ambient temperature recorded, °C.

25. Interpretation for Method D

25.1 Interpretation of the results is based on division into five-levels of 10°C increments of the compost heating (Refer to Table 05.08-D2). For example, Class I refers to 10°C, II is 20°C and the highest grade V is 50°C heating over ambient. Heating past this high point can occur but is unlikely owing to obvious self-limitation around 70°C. The results require about 2-9 days to record; fresh composts achieve elevated temperatures sooner than stable composts.

25.1.1 The five categories on the interpretation scale are often grouped by practitioners and European agencies into three major classes, where the lowest grade (I) is called "fresh-compost", the middle two (II-III) is referred to as "active compost", and the upper two (IV-V) are termed "finished compost". Compost marketers expect compost to be grade IV or V. The basis of this classification of ripeness is shown in Table

05.08-D1 Classification into five groups rather than three is arbitrary, and has been frequently debated in official circles. Essentially, the system has been upheld by more recent European work.

25.2 Some Dewar runs give inexplicable heat rise after a week or more in the vessel. Care must be taken to interpret the results of such anomalous samples. Woods End experience shows that heat or moisture damaged composts behave in this manner, appearing to be stable but re-heating significantly later, presumably due to re-establishment of indigenous microflora.

Table 05.08-D1 Example format for data collection.

Term	*Date/Time*	*Flask Temp. (°C)*	*Ambient Temp.(°C)*	*Net Rise (°C)*
Day 0				
Day 1				
Day 2				
Day 3				
Day 4... etc.				
			Maximum:	
			Stability Rating:	

Table 05.08-D2 Dewar self-heating increments, rating and description of stability classification based on the European system.

Temperature Rise Above Ambient	*Official Class of Stability*	*Descriptors of Maturity Class or Group*	*Major Group of Compost*
< 10°C	V	Finished Compost; stable to very stable compost	Finished
10° – 20°C	IV	Maturing; moderately unstable, curing compost	Curing
20° – 30°C	III	Active Compost, material decomposing and unstable	Active
30° – 40°C	II	Immature Compost, young or very active compost	Active
> 40°C	I	Raw Feedstock; fresh compost, mixed ingredients	Raw or Fresh

25.3 *Dewar Self-Heating versus CO_2 Respirometry*— Compost self-heating in a Dewar vessel is a respiration technique, and provides similar results to CO_2 respirometry measured over a 3-day to 7-day period. The Dewar test measures heat released during microbial respiration associated with the composting process. Table 05.08-D3 illustrates the relative relationships between heat rise measured in Dewar vessel and corresponding measures of CO_2 respiration levels.

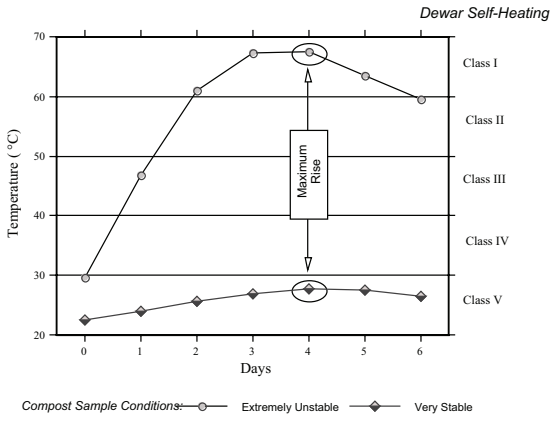


Fig 05.08-D1 Dewar Self-Heating illustration of test results for two distinct composts across a testing period of six days.

25.3.1 The data in the left and middle columns in Table 05.08-D3 indicate the respiration level and maturity classification. The right column of Table 05.08-D3 indicates the corresponding Dewar maturity classification that approximates accords to these levels.

It may be seen that Dewar saturates at a lower respiration rate than with CO_2 respirometry methods.

25.3.2 The difference between laboratory respiration and Dewar methods is that the Dewar vessel simulates heat take-off simultaneous with the experiment, and may quickly reach a self-limiting temperature, i.e., temperatures greater than 65°C. Only a very sophisticated laboratory feed-back apparatus which generates a heat rise proportional to carbon released during respiration could produce data which is truly comparable to the same as data obtained from a Dewar test.

25.3.3 The Dewar classification range is narrower than that provided by CO_2 respirometry alone. The Dewar method is driven by temperature increases induced through accelerated microbial respiration, most notably within Dewar classes III and IV. Unlike the Dewar self-heating procedure, conventional CO_2 or O_2 respirometric methods monitor microbial respiration at a fixed temperature in comparison to temperatures found in an actual composting conditions (Iannotti, 1993).

Table 05.08-D3 Relationship of CO_2 respiration to Dewar self-heating test and equivalent classes.

mg CO_2-C g TS d^{-1}	mg CO_2-C g OM d^{-1}	*Rating of Respiration*	*Equivalent Dewar Maturity Class*
0 – 6	0 – 4	▪ very low rate	V
6 – 25	4 – 16	▪ moderately low	IV-III
25 – 46	16 – 30	▪ medium rate	II-I
46 – 77	30 – 50	▪ medium-high rate	I
> 77	> 50	▪ high rate	

NOTE 1D—rating developed and used by Woods End scientists since 1980 and is based on screening several thousand composts

25.4 *Marketing Considerations*—European field data for biosolids composts suggests several ways to interpret the Dewar data. Table 05.08-D4 relates "Best Use" for source-separated residential food residue blended with yard-waste compost to the Dewar classifications. Different and possibly more conservative use guidelines may be applicable for other composts with different feedstocks.

25.5 *Pathogen Reduction*—The Dewar method may also be useful for assessing pathogen reduction. In one study, preliminary findings with biosolids composts suggest consistent pathogen removal by USEPA 40CFR503 standards after the compost achieves Dewar class IV and fails to re-heat after disturbance.

Table 05.08-D4 Proposed relationship of Dewar class to best use of compost.

Class of Stability Dewar Test	*Best Use of Compost Class*
V	Potting Mixes, seedling starters
IV	General Purpose Gardening, Greenhouse cultivation
III	All field Crops, Grapes, Fruit, Apples
II	Limited Field Cultivation with wait-period, (e.g., corn, tomatoes, broccoli, etc.)
I	Compost Raw Feedstock only

Test Method: Respirometry. Solvita® Maturity Index						**Units:** index		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.08-E	05.08-E	05.08-E		05.08-E		05.08-E

05.08-E SOLVITA® MATURITY INDEX

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTION—Dr. William F. Brinton, Woods End Research Laboratory, Inc.

26. Apparatus for Method E

26.1 *Solvita® jar*—sample container provided in the test kit package by the manufacturer.

27. Reagents and Materials for Method E

27.1 *Gel strips*—two color-coded paddles provided in the test Kit package by the manufacturer.

28. Procedure for Method E

28.1 *Transfer compost to the Solvita® jar*—Fill the jar to the fill line (Fig 05.08-E1). To obtain proper sample density, sharply tap the bottom of the jar on a counter. Fluffy or coarse composts should be compacted by pressing firmly into the jar.

Fig 05.08-E1 *Solvita®* test kit jar.

NOTE 1E—Equilibration Step. Compost in a sub-optimal state, as described under sample handling, may require equilibration for one to three d prior to testing. Equilibration may be necessary for thermophilic samples, for samples whose moisture is adjusted after collection, and for frozen samples.

28.2 *Remove gel paddles from their packaging*—Open each package by tearing along the top strip. Carefully remove each paddle by grasping its handle. Do not touch the special gel surface nor allow compost to physically contact the gel. Start the test within 30 min after the Solvita® gelpacks are opened.

28.3 *Insert the paddles into the sample*—Orient the two paddles as indicated by the color squares on the jar label. Labels must be seen through their respective viewing-window. Push the paddle tips into the compost to the bottom of the jar. The paddles should be positioned at right angles to each other. Edges of the paddles can touch each other in the middle of their gel strips without affecting the results. Be careful not to jostle or tip the jar. Do not use a paddle if the gel is dry or discolored. The gel color should be that same as the "Control Color" indicated on the respective color charts.

Fig 05.08-E2 Gel paddle.

CAUTION—The gel is not harmful to touch, but should not physically contact the mouth or eyes.

28.4 *Screw the lid tight*—Allow the sample to incubate for four h out of contact with direct sunlight at an ambient temperature of 20-25°C (68-77°F).

Fig 05.08-E3 Test period is four h.

28.5 *Read the gel color*—Read and interpret the *Solvita®* paddle colors four h after the lid is secured and the test is started. With the lid in place, view the paddle colors through their respective viewing windows at the side of the jar. Illuminate the paddles from the front with moderate-intensity, fluorescent lighting. Compare to the color charts provided with the kit, and record the color numbers that most closely match (Refer to Fig 05.08-E4).

Fig 05.08-E4 Color indicators.

NOTE 2E—The Solvita® test is based on a 4-h reading. Gel color may continue to change after the 4-h incubation period, so it is imperative that color interpretation be performed at 4-h to ensure proper interpretation. Color is ideally interpreted under bright fluorescent lighting.

29. Interpretation of Results for Method E

29.1 The *Solvita® Maturity Index* of the compost sample is determined in Table 05.08-E1 from the test result color numbers for CO_2 and NH_3 corresponding to the color charts (Fig 05.08-E4). This index value is used to determine the level of compost maturity with the use of Table 05.08-E2. For composts with low ammonia (chart value 4 or 5) the *Solvita® Maturity Index* is the same as the CO_2-color number. For high ammonia levels, the *Solvita® Maturity Index* will be less than it appears from the CO_2 result. The reason is that ammonia can inhibit microbial activity and interfere with the CO_2 test.

29.2 As compost ages, it normally goes from a fresh condition (*Solvita® Index* 1 to 2) to a mature state (*Solvita® Index* 7 to 8). This can take weeks to months, depending on the materials and method of composting. Table 05.08-E1 presents an overview of this aging process and shows how other tests that are used to characterize stability can be compared to the Solvita® test.

Table 05.08-E1 Solvita® Maturity Index Computation Table.

			Paddle C							
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Paddle A	**5**	Very Low NH_3	1	2	3	4	5	6	7	8
	4	Low NH_3	1	2	3	4	5	6	7	8
	3	Medium NH_3	1	1	2	3	4	5	6	7
	2	High NH_3	1	1	1	2	3	4	5	6
	1	Very High NH_3	1	1	1	1	1	2	3	4

Table 05.08-E2 Solvita® Maturity Index and other Indexes.

Solvita® Maturity Index	STAGE OF THE COMPOSTING PROCESS	MAJOR CLASS	Approximate Equivalencies[1]		
			Dewar	CO_2–C Rate	O_2 Rate
8	Inactive, highly matured compost, very well aged, possibly over-aged, like soil; no limitations for usage	FINISHED COMPOST	**V**	1	< 3
7	Well matured, aged compost, cured; few limitations for usage			2	5
6	Curing; aeration requirement reduced; compost ready for piling; significantly reduced management requirements	*Curing*		4	11
5	Compost is moving past the active phase of decomposition and ready for curing; reduced need for intensive handling	ACTIVE COMPOST	**IV**	6	16
4	Compost in medium or moderately active stage of decomposition; needs on-going management	*Very Active*	**III**	8	21
3	Active compost; fresh ingredients, requires intensive oversight and management		**II**	10	27
2	Very active, putrescible fresh compost; high-respiration rate; requires very intensive aeration and/or turning	RAW COMPOST	**I**	12	32
1	Fresh, raw compost; typical of new mixes; extremely high rate of decomposition; putrescible or very odorous material			> 15	> 40

[1] Interpretations provided by Woods End Research
Column 1: Dewar Self Heating test using standard Dewar Flask, grades as per interpretation (see TMECC Method 05.08-D)
Column 2: CO_2 Rate is the total mg CO_2-C evolved per g (OM) per day at 34°C (see TMECC Method 05.08-B)
Column 3: O_2 Respiration Rate (SOUR) as mg O_2 consumed per g (OM) per day (calculated from Column 2)

Test Method: Respirometry. Biologically Available Carbon						**Units:** mg CO_2-C g^{-1} OC d^{-1}		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.08-F	05.08-F	05.08-F		05.08-F		05.08-F

05.08-F BIOLOGICALLY AVAILABLE CARBON

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

CONTRIBUTION—***PROPOSED METHOD*** by Frank Shields; Soil Control Lab; Watsonville, CA

30. Apparatus for Method F

30.1 *CO_2 analyzer and integrator*—(e.g., FUJI Electrie and HP3393A or equal).

30.2 *Incubator*—capable of maintaining 36°C.

30.3 *Analytical balance*—with ±0.005 g precision.

30.4 *Air tank*

30.5 *CO_2 tank*

30.6 *Syringes*—250, 100 and 25 µL.

30.7 *Oven*—forced-air, capable of sustaining 70°C.

30.8 *Sieve*—stainless steel or plastic, 4-mm (#5) mesh.

30.9 *Crucibles*—porcelain, 70 cm^3.

30.10 *Volatile organic acid bottles*—40-mL, typically used in GC analysis, (e.g., I-Chem 40-mL clear VOA).

30.11 *Rubber stopper*—2 hole, #1, to fit VOA bottle listed above.

30.12 *Vinyl tubing*—4-mm i.d.

30.13 *T-fitting*—4-mm o.d.

30.14 *Timer*—2 h.

31. Reagents and Materials for Method F

31.1 *Plastic bags*—to cover plastic cups, (6 in. × 9 in.).

31.2 *Plastic cups*—500-mL, (e.g., Solo P-16, 16-oz).

31.3 *Sand*—quartz, #20, soaked in 1N HCl two h, DI water wash, Heated 500°C).

31.4 *Water*—deionized, minimum resistivity 17 MΩ·cm, minimum standard.

31.5 *Stock Nutrient Solution (Hoagland Solution)*—Dissolve potassium phosphate in approximately 750 mL of water. Add and dissolve the other chemicals in the order presented (below). Dissolve each chemical before adding the next. Warm water will speed up the process. Bring to 1 L with DI water. Dilute 5× for working solution.

31.5.1 *potassium phosphate*—KH_2PO_4, 1.4 g.

31.5.2 *potassium nitrate*—KNO_3, 5.7 g.

31.5.3 *calcium nitrate*—$5Ca(NO_3)2{\cdot}NH_4NO_3{\cdot}10H_2O$, 8.5 g.

31.5.4 *magnesium sulfate*—$MgSO_4{\cdot}7H_2O$, 4.3 g.

32. Procedure for Method F

32.1 *Sample Preparation:*

32.1.1 Screen an as-received moist compost sample through a 4-mm sieve.

32.1.2 Determine total solids and organic carbon content on a parallel aliquot. Use Method 04.01 Organic Carbon.

32.1.3 Transfer 10.0 to 20.0 g of compost to a 500-mL plastic cup.

32.1.4 Add 90 g of sand to the same cup and mix thoroughly.

32.1.5 Stir in 20 mL of nutrient solution.

32.1.6 *Blank*—Prepare sand plus nutrient solution in a second 500-mL plastic cup.

32.1.7 Place the plastic bag over the top of each prepared 500-mL sample cup and incubate at 35°C for three d.

32.2 *Experimental:*

32.2.1 The incubated sand/compost and blank are re-mixed in their cups.

32.2.2 Sub-samples are removed for total solids and organic carbon determinations.

32.2.3 Place 4 to 10 grams of prepared sample in each of five 40-mL VOA bottles; include both samples and blank.

32.2.4 Zero the timer.

32.2.5 A light flow of air is introduced into the VOA bottle #1 for 8 sec, which is then capped and shaken.

32.2.6 At fifteen sec, air is introduced to the VOA bottle #2 until all ambient air is replaced with

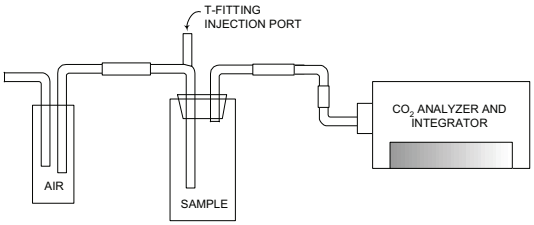
compressed air. The VOA bottle is then capped and the sample allowed to rest (respire) for one h.

32.2.6.1 *Calibrate the CO_2 Analyzer*—During the one-hour resting period, calibrate the CO_2 analyzer by injecting known concentrations of CO_2 into the T-fitting and plotting a line. The T-fitting is capped with tape between injections.

32.2.7 At exactly one h, the first VOA bottle is uncapped and quickly positioned in place of the sample VOA bottle #1 in the set-up. The CO2 produced in one h by that sample is recorded.

32.2.7.1 After fifteen sec the next sample VOA bottle is positioned in the setup to replace the previous sample. This process continues until all samples are measured.

32.3 *Preparation of CO_2 Apparatus:*



33. Calculations for Method F

33.1 Calculate the amount of CO_2-carbon (mg CO_2-C) per gram of compost organic carbon per day as follows:

$$X = (A \times 0.01286) \div OC \qquad \textit{Equation 33.1}$$

where:

X = mg CO_2-C g^{-1} OC d^{-1}

A = CO_2 per hour, µL, and

OC = organic carbon, % dw, determined using Method 04.01-A.

05.08 METHODS SUMMARY

34. Report

34.1 Report sample condition, including as-received moisture content, presence or absence of fungal mycelium, and sample color and odor as indicated in Method 05.08. Indicate whether sample is in an anaerobic or aerobic state upon testing, both before and after pre-incubation steps.

34.2 *Method 05.08-A Specific Oxygen Uptake Rate*—Report respiration rate as a function of O_2 consumption and the corresponding rating and characteristic, following the stability indexing system listed in Table 05.08-1; column 1 - SOUR OM.

34.2.1 *Units*—±1 mg O_2 g^{-1} TS d^{-1}, and

34.2.2 *Units*—±1 mg O_2 g^{-1} OM d^{-1}.

34.2.3 Report methods for determining mass units, TS and OM. Use Method 05.07 LOI Organic Matter for OM, sometimes referred to as BVS, as the unit mass and Method 03.09-A Total Solids and Moisture at 70±5°C for TS as the unit mass.

NOTE—OM represents the organic matter fraction of a sample and assumes that man-made volatile inerts, if present, do not exceed a sieve size of 4 mm.

34.3 *Method 05.08-C Carbon Dioxide Evolution Rate*—Report respiration rate as a function of CO_2 evolution and the corresponding rating and characteristic, following the stability indexing system listed in Table 05.08-1; column 2 - CO_2 Evolution.

34.3.1 *Units*—±1 mg CO_2-C g^{-1} OM d^{-1}, and

34.3.2 *Units*—±1 mg CO_2-C g^{-1} TS d^{-1}.

34.3.3 Report method for determining mass unit basis, TS or OM. Use Method 05.07 LOI Organic Matter for OM, sometimes referred to as BVS, as the unit mass and Method 03.09-A Total Solids and Moisture at 70±5°C for TS as the unit mass.

34.4 *Method 05.08-B In-Situ Oxygen Refresh Rate:*

34.4.1 *Units*—±0.5 %, % O_2.

34.4.2 Report description of apparatus and calibration technique employed at time of sampling.

34.5 *Method 05.08-D The Dewar Self-Heating*—Report method name and apparatus used; total solids content; source material of compost, (e.g., municipal solid waste, biosolids, yard waste, etc.); net temperature rise (Δ°C); maximum sample temperature (°C); ambient temperature (°C) under test conditions, the number of days (d) required to reach maximum temperature rise; and stability class as determined using Table 05.08-D2.

34.6 *Method 05.08-E The Solvita® Maturity Index*—Report on a scale of 1 to 8 determined using the 2-way table, Table 05.08-E1.

34.6.1 Report the relative CO_2 level using the *Solvita®* scale of 1 to 8.

34.6.2 Report the relative NH_3 level using the *Solvita®* scale of 1 to 5.

34.6.3 Report all sample pretreatment steps beyond those described under paragraphs 7.4 and 28 of this protocol, (e.g., extended incubation times, temperature equilibration, moistening of sample, drying of sample, etc.).

34.7 *Method 05.08-F Biologically Available Carbon:*

34.7.1 *Units*—±1 mg CO_2-C g^{-1} OC d^{-1}, where OC, is the organic carbon content determined using Method 04.01.

35. Interpretation of Results

35.1 The Compost Stability Index is based upon results of respiration monitoring to measure the relative level of microbial activity in a sample (Table 05.08-1).

35.2 The level of microbial activity in a sample is determined using results of respiration monitoring; however, the index (below) assumes optimized moisture, temperature and nutrient status that favor microbial activity, and insignificant concentrations of toxins and other compounds that inhibit microbial respiration.

35.3 Generally, it is not appropriate to report respirometry test results as the sole measure of compost stability. Always review analytical results for nutrient content, pH, electrical conductivity, etc., and screen for the presence of phytotoxins with a biological assay when establishing compost use guidelines or restrictions.

Table 05.08-1 Compost Stability Index—Ranges indicate relative compost stability for respiration methods described in TMECC. The level of microbial activity in a sample is based primarily upon results of respiration monitoring. The index assumes optimized moisture, temperature, pH, and nutrient status that favor microbial activity, and insignificant concentrations compounds that inhibit microbial respiration.

SOUR (OM) 05.08-A	*CO_2-C 05.08-B*	*DEWAR 05.08-D*	*SOLVITA® 05.08-E*	*BAC OC 05.08-F*	*STABILITY RATING*	*GENERAL CHARACTERISTICS*
< 3	1	V	8	< 2	very stable	▪ well cured, finished compost ▪ no continued decomposition ▪ no odors ▪ no potential for VFA phytotoxicity and odor
3 – 10	2 – 4		7	2 – 4	stable	▪ moderately well cured compost ▪ odor production not likely ▪ limited potential for VFA phytotoxicity and odor ▪ minimal to no impact on soil carbon and nitrogen dynamics
11 – 20	5 – 7	IV	5 – 6	5 – 8	moderately unstable, curing compost	▪ curing compost ▪ odor production not likely ▪ aeration requirement reduced ▪ limited potential for VFA phytotoxicity and odor ▪ minor impact on soil carbon and nitrogen dynamics
21 – 26	8 – 9	III	4	9 – 12	unstable raw compost	▪ active, uncured compost ▪ minimal odor production ▪ high aeration requirement ▪ moderate to high potential for VFA phytotoxicity ▪ moderate potential for negative impact on soil carbon and nitrogen dynamics
27 – 31	10 – 11	II	3	13 – 20	raw compost, raw organic products	▪ highly active, uncured compost ▪ odor production likely ▪ high aeration requirement ▪ high potential for VFA phytotoxicity and odor ▪ high potential for negative impact on soil carbon and nitrogen dynamics
> 32	> 11	I	1 – 2	> 20	raw feedstock, unstabilized material	▪ raw, extremely unstable material ▪ odor production expected ▪ high aeration requirement ▪ probable VFA phytotoxicity with most materials ▪ negative impact on soil carbon and nitrogen dynamics expected ▪ generally not recommended for use as compost

REPORTING UNITS:
SOUR OM : mg O_2 g^{-1} OM d^{-1}; *CO_2-C :* mg CO_2-C g^{-1} OM d^{-1}; *Dewar and Solvita® :* refer to respective indices; *BAC OM* : mg CO_2-C g^{-1} OC d^{-1}. It is not recommended to report a respirometry test result as the sole measure of compost stability.

NOTE—Anticipate refinement of the compost stability index with advances in compost stability research.

36. Precision and Bias

36.1.1 *Method 05.08-A Specific Oxygen Uptake Rate*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

36.2 *Method 05.08-B Carbon Dioxide Evolution Rate*—The precision and bias of this test are not documented. Data are being sought for use in developing a precision and bias statement.

36.2.1 Precision of the titrimetric method is approximately ±10% of the known CO_2 concentration.

36.2.2 *Method 05.08-C In-Situ Oxygen Refresh Rate*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

36.3 *Method 05.08-D The Dewar Self-Heating Method*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

36.4 *Method 05.08-E Solvita® Maturity Index*—Color change is linear over the range of CO_2 or NH_3 tested. The tonal range of the color indicator is accurate even for color-blind perception. Half-tone color changes can be accurately interpolated by the trained eye.

36.5 *Method 05.08-F Biologically Available Carbon*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

37. Keywords

37.1 carbon dioxide (CO_2) evolution; microbial activity; oxygen (O_2); refresh rate; respiration; stability; specific oxygen uptake rate; SOUR; respirometry; self-heating; Dewar; Solvita®; ammonia; biologically available carbon

Test Method: Viable Weed in Compost. Two Methods						**Units:** count $(4 \cdot L)^{-1}$		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
						05.09-A		05.09-A
						05.09-B		05.09-B

05.09 VIABLE WEED IN COMPOST

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This method covers tests to determination the quantity of weed seed and germinable plant parts per volume of compost.

1.1.1 *Method 05.09-A Shields Rinse Method.*

SUBMITTED BY—Frank Shields - Soil Control Laboratory

1.1.2 *Method 05.09-B Peat Moss Dilution Method.*

SUBMITTED BY—Dr. William F. Brinton, Jr., Woods End Research Laboratory, Inc.

1.2 The values stated in SI units are to be regarded as the standard. The values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 02.01 Field Sampling of Compost Materials.

Method 03.09 Total Solids and Moisture.

Method 04.10 Electrical Conductivity.

Method 04.11 Electrometric pH Determination.

Method 05.05 Biological Assays.

2.2 *Other References:*

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Shiralipour, A., and D. B. McConnell. 1991. Influence of yard trash composting on weed tree seed germination. Proc. for Envronmentally Sound Agric. Conf. Vol. 2. April 6-18, 1991. Orlando, FL.

3. Terminology

3.1 *botanical composition, n*—The compositional makeup of weed type as presented by species. For botanical classification, it may be necessary to allow weeds to grow-out for up to 6 weeks in the test media.

3.2 *dormancy, n*—The condition or state of seeds of many non-cultivated species, which do not sprout when first, exposed to adequate moisture, temperature and oxygen. Dormancy is generally broken by environmental factors and is species-specific. Freezing, scarification, flash drying and re-wetting, exposure to red light and prolonged cool ambient temperatures are factors known to break the dormancy cycle of specific weed species.

3.3 *germination inhibition, n*—Any of several conditions induced through the occurrence of one or many edaphic factors at levels that diminish the viability of a seed; common factors include salt concentration, volatile organic acids and adequacy of oxygen; i.e., maturity in compost.

3.4 *media dilution, n*—The dilution on a volume basis (v/v) with any compost with a standard agent, such as

peat moss to achieve standard conductivity volume (SCV).

3.5 *germinable plant part, n*—Any portion of a plant, rootlet or stem capable of vegetative reproduction; i.e., rhizome, etc.

3.6 *viable weed, n*—A germinable weed seed. A weed seed capable of germinating under optimized conditions in the test sample. Does not include dormant weed seeds.

3.7 *weed seed, n*—undesirable viable seed.

4. Summary of Test Method

4.1 *Method 05.09-A Shields Rinse Method*—A known and representative volume of compost prepared by repeated soaking in deionized water until the soluble salts and readily available carbon compounds are reduced to non-toxic concentrations. The prepared compost sample aliquot is filtered and spread onto a layer of sand and incubated at 20ºC for one week. Emerged weed seedlings are counted, recorded and removed. The number of weed seedlings recorded is extrapolated to represent the entire batch or pile and is reported as the number of viable weed seeds per 4 L of compost.

NOTE 1—Readily available carbon compounds are present in immature composts, but not present at significant concentrations in mature, properly managed composts.

4.2 *Method 05.09-B Peat Moss Dilution Method*—A quantitative procedure which standardizes the conductivity of compost by dilution with limed peat. The test determines the content of viable weed seeds and germinable plant parts and reports them per 4 L of test material. The test requires approximately 14 days and compost diluted to a standard conductivity volume. The test provides an interpretation scale for judging results.

5. Significance and Use

5.1 Properly prepared compost is relatively weed seed free. The presence of weed seed in a compost indicates that the material may not have been adequately processed or that the finished compost was contaminated from the air, or stored on or mixed with material containing weed seed.

5.2 A weed free compost reduces time and expense of weeding and is a more marketable product. Even though weed seeds might be deposited onto the surface of curing piles, the presence of significant numbers of viable weed seeds in compost might indicate that adequate heating was not attained during the thermophilic phase of the composting process.

5.3 Determination of weed seed content may improve understanding of weed infestations that arise from composts and potting media. The origin may be hypothesized by examining the botanical composition of the weed species present within the region of a composting facility.

5.4 Viable weed seed determination is useful for product quality assurance in specialized markets that are sensitive to weeds in substrates, (e.g. horticultural and mulch).

6. Interference and Limitations

6.1 The aliquot used with this test is assumed to accurately represent the compost bulk in question. Sample size can be a limiting factor. Consider the sample collection issues presented in TMECC Section 02.01.

6.2 Excessive salts, the presence readily available organic carbon that can produce anaerobic conditions and other phytotoxins that suppress seedling germination must be diluted to nil or eliminated to facilitate viable seed germination.

6.2.1 Salts interfere with germination of many weed seeds with wide variation among species. To remove this interference, dilution to a conductivity of less than 2 dS m^{-1} is generally considered adequate. The sensitivity of specific weed seeds may have to be determined in advance.

6.3 This test does not purport to address the various factors that break dormancy of weed seeds.

6.3.1 The presence of dormant seed is not specified in the sample preparation section for this test. Edaphic factors that break seed dormancy vary with plant species. It may be necessary to replicate this test for each seed dormancy factor that is identified for the region of interest. If necessary, appropriate steps should be devised to break seed dormancy for the species of interest.

6.3.2 This test was devised for use on composts applied to the receiving soil in the current growing season. If the compost application is to be applied in the fall with plans for planting in the spring it may be best to cycle freezing/thawing the compost sample before testing to simulate the climate conditions. It may be necessary to introduce steps that emulate conditions of other climates.

6.4 Nutrients and other factors present in organic amendments may enhance the germination and growth of some weed seeds already present in soils. Field trials are recommended if weed problems persist, despite test results.

6.5 Even with the use of a positive control with radish, there is no known means to interpret it. If radish germinates poorly in standardized mix, it may be an

indication of phytotoxic factors present that have not been eliminated by the proposed dilution. It is not known if those factors would affect weed seeds to the same extent as they do radish.

7. Sample Handling

7.1 *Method 05.09-A Shields Rinse Method*—Sample size is four liters (4·L).

7.1.1 Store compost samples at as-received moisture at 4ºC until test is started. Avoid freezing the sample, especially when the sample source has a climate where freezing rarely occurs.

7.1.2 Store compost samples intended for this test at 4ºC for no more that seven days. Seven-day refrigerated storage is assumed to be the maximum allowable storage time for samples that are destined for biological analysis.

7.2 *Method 05.09-B Peat Moss Dilution Method*—Compost is sampled to include all representative fractions as described in *Method 02.01-C Selection of Sampling Locations for Windrows and Piles*. It may be advisable to sample the windrow surface (0-15 cm) separately from the windrow core, specifically when infestations of weeds from air-borne sources are observed to occur. Similarly, samples from bagged commercial products should observe cautions described in Table 02.01-A1 *Sampling operations, constraints and required tools for ten types of composting technologies*.

7.2.1 Peat is obtained and prepared with limestone a minimum of one week prior to testing.

7.2.2 Large volume trays must be available to accommodate the potentially large dilutions.

Test Method: Viable Weed in Compost. Shields Rinse Method						**Units:** count $(4 \cdot L)^{-1}$		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
						05.09-A		05.09-A

05.09-A SHIELDS RINSE METHOD

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

SUBMITTED BY—Frank Shields - Soil Control Laboratory; Watsonville, CA 75076 USA; frank@compostlab.com

8. Apparatus for Method A

8.1 *vacuum*—wet/dry, 5-gal capacity.

8.2 *bucket*—plastic, 5-gal.

8.3 *pail*—plastic, 3-gal, with bottom removed.

8.4 *incubator*—capable of maintaining a temperature of 20ºC.

8.5 *electrical conductivity meter*.

8.6 *screen mesh*—plastic, 300-µm mesh, secured to the bottom of the 3-gal plactic bucket.

8.7 *trays*—plastic, 10 cm (4 in.) high, with cumulative surface area of 4500 cm^2 (700 $in.^2$).

9. Reagents and Materials for Method A

9.1 *sand*—quartz, # 20.

9.2 *water*—deionized, minimum resistivity 17 MΩ·cm, minimum standard.

10. Procedure for Method A

10.1 *Prepare plastic box:*

10.1.1 Transfer sand to the plastic box. Form a level 2.5-cm (1-in.) thick layer of sand across the bottom of the plastic box.

10.2 *Remove salts and soluble organics from the compost test sample:*

10.2.1 Transfer 4 L of compost to a 20-L (5-gal) bucket.

10.2.2 Add 16 L of deionized water to the compost sample and thoroughly mix. Measure the electrical conductivity of the mixture.

10.2.3 Place the plastic bucket with screen mesh bottom into the compost water slurry and move it up and down to water will enter pipe. Remove as much water as practical using the wet/dry vacuum.

10.2.4 Repeat steps 10.3.2 and 10.3.3 until the electrical conductivity is below 0.30 dS m^{-1} (mMhos cm^{-1}). (about 3 to 4 times)

10.3 Spread a 2 cm layer of the prepared (salt and organics-reduced) compost across the top of the sand in the plastic box.

10.4 *Introduce positive control*—Distribute ten radish seeds. across the prepared compost test sample at a known, recorded locations in the box.

10.5 Cover the box with a transparent cover to minimize evaporative water loss. Record the sample identifier and date on the side of the box for future reference.

10.6 Maintain the prepared box at 20ºC for approximately three weeks.

10.7 Monitor daily.

10.7.1 Count and remove radish seeds as they germinate.

11. Calculations for Method A

NOTE 2A—Replication using multiple samples from one compost production batch or windrow may be necessary to accurately estimate the incidence of weed seed in compost. The statistical validity of this method has not been determined. Randomly extracted aliquots should be used as replicates to approximate the relative accuracy and reliability of test results.

11.1 Calculate percent viability of radish seed control:

$$C = R \div 10 \times 100 \qquad \textit{Equation 11.2}$$

where:

C = percent of viable seeds for control,
R = number of viable control seeds,
10 = initial number of control seeds planted, and
100 = factor to convert ratio to percentage.

Test Method: Viable Weed in Compost. Peat Moss Dilution Method						**Units:** count $(4{\cdot}L)^{-1}$		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.09-B		05.09-B				05.09-B

05.09-B PEAT MOSS DILUTION METHOD

SUBMITTED BY—Dr. William F. Brinton, Jr., Woods End Research Laboratory, Inc.

12. Apparatus for Method B

12.1 *seedling flats*—standard plastic planting trays, size: 25 cm × 50 cm (10 in. × 20 in.). Two to ten are used for each trial, the quantity being proportional to the conductivity. Trays should be fitted with transparent tray covers that permit aeration and minimize evaporative water loss during germination. Alternatively, trays are placed uncovered in a growth chamber of controlled humidity of 70±5%. The test blend will be 1.25 cm deep in trays; the quantity of required trays may be calculated for any standard conductivity:

$$X = EC \div (2 \div DSC) \qquad \textit{Equation 12.1}$$

where:

X = required number of trays;
EC = conductivity in dS m^{-1}, and
DSC = target diluted standard conductivity (assumed in this method to be 2 dS m^{-1}). For practical purposes, if the method is used at the suggested target EC of 2 dS m^{-1}, then the number of required trays is equal to the conductivity, but never less than two trays.

12.2 *lighting*—grow-light conditions providing a minimum of 25000 lumens m^{-2} at the media surface. Day length shall be 12 h to 16 h. Night light conditions should be tungsten 45 Watt (ISO, 1993).

12.3 *radish seeds*—Untreated seed, germination certified. Source: Johnny's Selected Seeds Albion Maine, 04910 (or equivalent source). Alternatively, barley *Hordeum vulgare*) may be used as it is a standard test plant for phytotoxicity trials (ISO, 1993).

13. Materials for Method B

13.1 *sphagnum peat moss*—untreated (not fertilized, lime-free), light undecomposed moss peat, Van Post scale H 1-3; proper sphagnum moss contains 75% by dry weight of sphagnum fibers and a minimum of 90% organic matter by loss-on-ignition. Source: Fafard or Premier peat, or equivalent

13.2 *limestone*—finely ground agricultural grade limestone, $CaCO_3$ with a minimum of 32% Ca, or dolomitic limestone $CaMg(CO_3)_2$ with minimum of 20% Ca and 12% Mg, Fineness of grind: 98% should pass a 20 mesh and 40% should pass a 100-mesh; material courser than this requires considerably longer to equilibrate with peat. Source: Lee Limestone, Lee, MA or equivalent.

14. Procedure for Method B

14.1 Prepare standard limed peat by adding 12.6g limestone per 1000 cm^3 peat moss, or other rate determined empirically to bring about the requisite pH change. This pre-mix should be allowed to stand for not less than one week prior to use to allow equilibration. The final pH of the peat base should be 6.2±0.5 (Method 08.11-A). If the desired pH is not achieved, repeat the operation with the same material using smaller lime increments, repeating also the pH test procedure. This limed peat base is stable and may be stored for three months. Repeat pH tests every three weeks of storage, or within one week of any batch of testing.

14.2 Measure and record the electrical conductivity of the test compost (Method 04.10-A).

14.3 *Dilution of Compost Media*—Dilute compost on a volume basis as follows: based on the conductivity of the test compost, dilute with peat to arrive at a target of 2 dS m^{-1} (mmhos cm^{-1}) in the final mix. Use Equation 15.1 to determine the volume of peat required for dilution.

14.4 Lay sieved (<9.5 mm) compost + peat blend in trays to a depth of 1.25 cm (½ in.) and bring to field capacity with water, i.e., approximately 70% of WHC. Re-moistening trays with proper set-up should be unnecessary in the first 3 to 4 d. Temperature should be maintained at 20ºC to 22ºC for the duration of the experiment. Examine trays daily and maintain field capacity moisture status, misting with water when needed to keep the compost surface moist.

14.5 Tally weeds that have emerged at end of week two and report the number of viable weeds and germinable plant parts per 4·L of compost.

14.6 *Positive Control*—Prepare two positive controls. Add 25 each of radish (*Rhaphanus sativus*) in rows 1"

apart to each of two trays prepared with standard limed peat and with the prepared compost/peat blend. If compost has not been diluted, then a peat tray is unnecessary. Cover with one cm of substrate (either peat or compost/peat mix). Count radish emergence after five days. Acceptable germination: The two controls should agree with each other within 10±2 %. Acceptable response of radish in test media with compost is considered to be 90±5% multiplied by the tested germination rate of seed, imprinted on the seed package. If germination percentage of radish is significantly less than the peat control, it may be advisable to repeat test procedure at another higher dilution ratio of compost. If both controls (peat and peat+compost) give poor results, repeat test with new supply of test seeds.

15. Calculations for Method B

15.1 Calculate required dilution of compost media as ratio of peat to compost in compost + peat blend

$$X = (B - 2) \div (2 - C) \qquad \textit{Equation 15.1}$$

where:

X = Ratio of peat moss to compost in the compost/peat blend, v/v basis, (e.g., 1.0 means one part peat to each part compost, or a 50% dilution),

B = conductivity of compost before dilution, in dS m^{-1}, and

C = conductivity of peat prior to conducting the test.

05.09 METHODS SUMMARY

16. Report

16.1 *Method 05.09-A Shields Rinse Method*

16.1.1 Report the number of viable weed seeds per four liters (4·L) of compost.

16.1.2 Report the percent recovery for the radish seed control.

16.1.3 Report test conditions, i.e., pre-test storage and temperature cycling, incubation temperature, and duration of test.

16.1.4 Specify weed seed dormancy factors employed to break seed dormancy.

16.2 *Method 05.09-B Peat Moss Dilution Method*

16.2.1 Report as the number of viable weed seeds per four liters (4·L) of compost;

16.2.2 Report the germination rate of positive control in peat and peat+compost blend; and

16.2.3 Interpretation of quantity of weed seed may be conducted based on the suggested standard for horticultural methods, as indicated in Table 05.09-1.

Table 05.09 Interpretation guide for the presence of weed seed in compost, number of weeds $(4{\cdot}L)^{-1}$.

Weed Seeds per 4·L	*RATING*	*Suggested Use of Compost*
< 1	Weed-Free	Unlimited for potting and growing media
< 3	Very Low weed infestation	Mulch and general garden
3 - 7	Moderate infestation	Field use only
> 7	Significant Weed Infestation	Field use only [1]

SOURCE—Modified after LAGA-10 German Official Compost Test Methods. Bundesgutegemeinschaft Kompost e.V. Bonn. 1994.

17. Precision and Bias

17.1 *Method 05.09-A Shields Rinse Method*—The precision of this test is less than one weed per four liters [4·L] of compost. Bias of this test has not been determined. Data are being sought for use in developing a bias statement.

17.2 *Method 05.09-B Peat Moss Dilution Method*—The precision of this test is less than one weed per four liters [4·L] of compost. Bias had not yet been determined.

18. Keywords

18.1 weed seed; viable seed; germination; emergence; dormancy; viable weeds; moss; peat

1 Field Use compost usage is not meant to imply weed seeds present in this amount are acceptable in any application. The actual species composition and the usage should be carefully examined in each case.

Test Method: Volatile Fatty Acids. One Method							**Units:** mmoles g^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.10-A	05.10-A	05.10-A				05.10-A

05.10 VOLATILE FATTY ACIDS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This test covers the determination of volatile fatty acids content of compost.

1.1.1 *Method 05.10-A Volatile Fatty Acids in Compost Extract by Gas Chromatography.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

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Zijlistra, J.B., J. Beukema, B.G. Wolthers, B.M. Byrne, A. Groen and J. Dankert. 1977. Pretreatment methods prior to gas chromatographic analysis of volatile fatty acids from faecal samples. Clinica Chimica Acta. 78:243-250.

3. Terminology

3.1 *acetic acid, n*—Clear, colorless organic acid, CH_3COOH (mol wt 60.05), with a distinctive pungent odor, used as a solvent and in the manufacture of rubber, plastics, acetate fibers, pharmaceuticals, and photographic chemicals. It is the chief acid of vinegar.

3.2 *butyric acid n*—Either of two colorless isomeric acids, C_3H_7COOH (mol wt 88.11), occurring in animal milk fats and used in disinfectants, emulsifying agents, and pharmaceuticals. Also called butanoic acid.

3.3 *caproic acid, n*—A liquid fatty acid, $CH_3(CH_2)_4COOH$ (mol wt 116.16), found in animal fats and oils or synthesized and used in the manufacture of pharmaceuticals and flavorings. From Latin caper capr-goat (from the acid's goatlike smell).

3.4 *fatty acid, n*—Any of a large group of monobasic acids, especially those found in animal and vegetable fats and oils, having the general formula $C_nH_{2n+1}COOH$. Characteristically made up of saturated or unsaturated aliphatic compounds, this group of acids includes palmitic, stearic, and oleic acids.

3.5 *formic acid, n*—A colorless caustic fuming liquid, HCOOH (mol wt 46.03), that is extremely irritating to the skin: it is found in ants, spiders, etc. as well as in nettles and some other plants, used as a food preservative in dyeing and finishing textiles and paper, and in the manufacture of fumigants, insecticides, and refrigerants.

3.6 *heptanoic acid, n*—Enanthic acid, oenanthic acid, oenanthylic acid, *n*-heptoic acid, *n*-heptylic acid; $CH_3(CH_2)_5COOH$ (mol wt 130.19); oily liquid; disagreeable rancid odor; faint tallow-like color when spectroscopically clear; found in various fusel oils in

appreciable amounts; has been observed in rancid oils; boiling point: 223.01°C.

3.7 *isobutyric acid*—Either of two colorless isomeric acids, C_3H_7COOH (mol wt 88.11), occurring in animal milk fats and used in disinfectants, emulsifying agents, and pharmaceuticals. Also called butanoic acid.

3.8 *isovaleric, n*—Either of two colorless isomeric acids, $C_5H_{10}O_2$ (mol wt 102.13), used in flavorings, perfumes, plasticizers, and pharmaceuticals. Derived from the plant valerian for its occurrence in the plant's root.

3.9 *lactic acid, n*—A yellowish or clear, syrupy organic acid, $C_3H_6O_3$ (mol wt 90.08), produced by the fermentation of lactose when milk sours or from sucrose and some other carbohydrates by the action of certain microorganisms, and used in tanning leather, as a preservative, in the formation of plasticizers, etc.

3.10 *propionic acid, n*—A liquid fatty acid, CH_3CH_2COOH (mol wt 74.08), found naturally in sweat, in milk products, and as a product of bacterial fermentation. Prepared synthetically from ethyl alcohol and carbon monoxide, it is used chiefly in the form of its propionates as a mold inhibitor in bread and as an ingredient in perfume. Also called propanoic acid.

3.11 *valeric acid, n*—A colorless liquid, $C_5H_{10}O_2$ (mol wt 102.13), used in flavorings, perfumes, plasticizers, and pharmaceuticals.

3.12 *volatile, adj*—Evaporating readily at normal temperatures and pressures.

4. Summary of Test Methods

4.1 *Method 05.10-A Volatile Fatty Acids in Compost Extract by Gas Chromatography*

4.1.1 A compost sample is incubated in phosphate buffer for 1 h. Samples of the aqueous solvent are extracted from the sample, acidified and analyzed with a gas chromatograph. The quantitative determination of each volatile fatty acid (VFA) and the total VFAs in each sample are determined by comparison with standard solutions of VFAs. Before gas chromatography analysis, the sample is pretreated by vacuum distillation or by passing it through a cation exchange column.

5. Significance and Use

5.1 Volatile Fatty Acids (VFAs) form during bacterial decomposition of complex organic material, and during conditions of anaerobic activity. They are produced in large intestine of mammals, including humans when dietary fibers, unabsorbed sugars and starch are fermented. They are described as being volatile since they can be steam distilled under acidic conditions, and are also classified as being short chain fatty acids (less than C_8) because of their low molecular weight. Volatile Fatty Acids are unstable compounds and are converted during aerobic decomposition to form carbon dioxide and water, and during anaerobic decomposition to form carbon dioxide and methane.

5.2 Volatile fatty acids include formic acid (C_1), acetic acid (C_2) propionic and lactic acid (C_3), isobutyric and n-butyric acids (C_4), isovaleric and n-valeric acids (C_5), isocaproic and n-caproic acids (C_6) and n-heptanoic acid (C_7). VFAs are a source of odors during composting, especially acetic acid. VFAs can be phytotoxic and inhibit or delay seed germination and plant growth, but fortunately VFAs can be readily degraded in compost under aerobic conditions.

5.3 The amount of volatile fatty acids within a compost sample is not constant and depends upon the relative stability, porosity, particle size (aggregate size), and the availability and distribution of oxygen within compost. The surface area exposed to oxygen increases as compost matures. Particles of immature compost degrade aerobically when sufficiently high concentrations of oxygen are maintained across the aggregate surface. Volatile fatty acids will form most readily within aggregates where anaerobic conditions dominate. As degradation progresses, particle size decreases and aerobic conditions prevail when oxygen supply and aeration are adequate. VFA production then diminishes and those present degrade to form CO_2 and H_2O. When fresh inoculum is added to stable compost, the concentration of VFAs is likely to increase because of increased microbial activity within the larger immature aggregates.

6. Sample Handling

6.1 *Method 05.10-A Volatile Fatty Acids in Compost Extract by Gas Chromatography*—Compost aliquots at as-received moisture are hand sorted to remove particles of wood, glass, metal and hard plastics and homogenized in a blender for 30 seconds.

Test Method: Volatile Fatty Acids. VFA in Compost Extract by Gas Chromatography							**Units:** mmoles g^{-1}	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
		05.10-A	05.10-A	05.10-A				05.10-A

05.10-A VOLATILE FATTY ACIDS IN COMPOST EXTRACT BY GAS CHROMATOGRAPHY

LOOK—Sampling Handling issues are presented as part of the introduction to this section.

7. Apparatus for Method A

7.1 Refer to Table 05.10-A1 for gas chromatographic conditions used to measure volatile fatty acids (VFAs).

7.1.1 A gas chromatograph (GC) consists of an injection port, a glass column packed with an adsorbent and placed in an oven, and a detector. The output from the detector is either connected to a chart recorder and/or interfaced to a computer.

7.1.2 For GC analysis of VFAs the temperature of the injection port must be greater than the boiling point of the VFAs as the sample must be in the gas phase when flowing through the column.

7.1.3 During analysis the oven temperature is isothermal for SP 1200 packing materials and changes with time when the Chromosorb 101, Anakrom Polyester and Carbowax packing materials are used for analysis (refer to Table 05.10-A1).

Table 05.10-A1 Gas chromatographic conditions for VFA measurements.

Stationary Phase	*Column Length (m)*	*Column internal diameter (mm)*	*Oven Temp (°C)*		*Injector Temp (°C)*	*Detector Temp (°C)*	*Carrier Gas and flow rate (mL/m)*	*Sample Size (µL)*
			Initial	*Final*				
5% neopentyl glycol sebacte plus 1% H_3PO_4 on Anakrom Polyester	2	2	100	130	180	180	OFN, 40	1.0
SP 1200	1.83	2	125	125	200	200	N_2, 40	1.0
Carbowax	0.91	2	110	190	150	200	N_2, 20	1.0
Chromosorb 101	2.00	2	140	215	200	200	N_2, 15-20	5.0

7.1.3.1 *Anakrom polyester*—Oven is at 100°C for 2 min after which it is programmed to increase to 150°C at a ramp rate of 30°C min^{-1}.

7.1.3.2 *Carbowax*—Oven is at 110°C for 1.5 min after which it is programmed to increase to 190°C at a ramp rate of 20°C min^{-1} and the oven is held at this temperature for 4.5 min.

7.1.3.3 *Chromosorb 101*—Oven is at 140°C for 3 min after which it is programmed to increase to 215 at a ramp rate of 5°C min^{-1} and the oven is held at this temperature for 3 min.

7.1.4 A flame ionization detector is used to detect VFAs.

NOTE 1A—The flame ionization detector does not respond to formic acid, HCOOH.

7.1.5 The gaseous VFA samples flow through from the injection port to the detector with the aid of an inert gas, the *carrier gas.* For example, analar grade N_2, oxygen free nitrogen (OFN), and H_2 are typical gases.

7.1.6 Gas chromatography separates mixtures of components by partitioning the gases between a *stationary solid phase* and a *moving gaseous phase*, the carrier gas. Separation of mixtures of the VFAs is achieved by the selective absorption of the VFAs within the stationary bed. The weaker the interaction between the stationary phase and VFA the faster a VFA will move through a column. The strength of the interaction for a homologous series like the VFAs depends upon the molecular weight of the acid. VFAs of lower molecular weight interact less with the stationary phase than acids of higher molecular weight. The VFAs elute from a column in ascending molecular

weight. Separation of the isomers, (e.g., butyric versus isobutyric), of the VFAs is possible with GC. For the columns used in Table 05.10-A1 isobutyric, isovaleric and isocaproic acids should elute before butyric acid, n-valeric and n-caproic acids, respectively.

7.2 Vacuum distillation equipment is required for pretreatment of sample prior to GC analysis.

7.2.1 The equipment is shown in Figure 05.10-A1 It consists of a specimen flask connected via glass tubing to a receiver tube with the spout of the receiver tube connected to a water suction pump. Both the specimen flask and the receiver tube are sealed with Sovirel joints. The specimen flask is placed in an oil bath (20 to 120°C) and the receiver tube is cooled in liquid nitrogen (-196°C). The oil bath is placed on a magnetic stirrer.

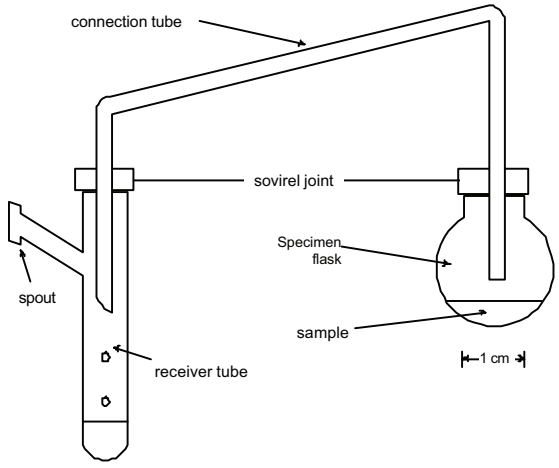

Fig 05.10-A1 Schematic of apparatus for vacuum distillation.

8. Reagents and Materials for Method A

8.1 *Water*—Type II deionized, 17 M? ·cm minimum standard.

8.2 *Hamilton Syringe*—(1-µL) for injection of samples into GC. Before use and between injections, the syringe is washed with water, ethanol and ethyl ether and dried by suction.

8.3 *Phosphate Buffer*—(0.2 mol L^{-1}), pH 7.0. Add 3 L of water to 5-L volumetric flask and dissolve 174.513 g of K_2HPO_4 in the water. Add more water to make a final volume of 5 L. Test pH with glass-electrode pH meter, adjust the pH with 5 M NaOH until a pH of 7.0 is attained.

8.4 *Formic Acid*—Add 1 mL of formic acid to 1 L of water to make a 0.1% vol/vol solution of formic acid in water.

8.5 *Standard Solution*—containing 10 mmoles L^{-1} of all VFAs.

8.5.1 Add 500 mL of phosphate buffer to a 1-L volumetric flask and dissolve 0.48 g of formic acid, 0.60 g of acetic acid, 0.741 g of propionic acid , 0.881 g of isobutyric acid, 0.881 g of n-butyric acid, 1.02 g of isovaleric acid, 1.02 g n-valeric acid, 1.161 g of isocaproic acid, 1.161 g n-caproic acids and 1.302 g of n-heptanoic acid in this solution. Add more phosphate buffer (approximately 452 mL) to make a final volume of 1 L.

8.6 *Standard Calibration Solutions*—containing all VFAs. The standard solution that contains all VFAs is diluted 2, 10 and 100 fold to make three solutions containing 0.1, 1 and 5 mmoles L^{-1} of each VFA. All solutions are further diluted in 0.1 % formic acid to make standard calibration solutions containing 9, 4.5, 0.9 and 0.09 mmoles L^{-1} of each VFA.

8.6.1 Add 50 mL of phosphate buffer to 50 mL of standard solution and mix in a 100-mL volumetric flask. This is the two-fold dilution, 5 moles L^{-1}.

8.6.2 Add 90 mL of phosphate buffer to 10 mL of standard solution and mix in a 100-mL volumetric flask. This is the ten-fold dilution, 1 moles L^{-1}.

8.6.3 Add 1 mL of phosphate buffer to 99 mL of standard solution and mix in a 100-mL volumetric flask. This is the one hundred-fold dilution, 0.1 moles L^{-1}.

8.6.4 Place 0.9 mL of each standard solution (10, 5, 1 and 0,1 moles L^{-1}) into a glass vial and add 0.1 mL of 0.1% formic acid.

8.7 *Standard Solution Containing only One VFA*—at 9 moles L^{-1} in formic acid. This section is *only* useful when qualitative measurements are required.

8.7.1 Add 50 mL of 0.2 mol L^{-1} phosphate buffer to a 100-mL volumetric flask and dissolve 0.060 g of acetic acid. Add more phosphate buffer to make a final volume of 100 mL.

8.7.2 Repeat step 8.5.1 and make standard solutions of propionic acid (0.0741 g), isobutyric and n-butyric acids (0.0881 g) isovaleric and n-valeric acids (0.102 g), isocaproic and n-caproic acids (0.1161 g) and n-heptanoic acid (0.1302 g) in phosphate buffer.

8.7.3 Place 0.9 mL of all solutions into a glass vial and add 0.1 mL of 0.1% formic acid.

8.8 *Optional*—Pasteur pipettes filled with 1 mL of cation-exchange resin (AG 50 W-X_4; 200-400 mesh).

8.8.1 Wash 10 mL of resin with distilled water until the supernatant is clear.

8.8.2 Wash resin with excess 1 *M* NaOH for 24 h and then neutralize it to pH 7 with distilled water.

8.8.3 Wash resin with 4 *M* HCl for 4 h.

8.8.4 Neutralize resin to pH 7.0 by repeated washing with distilled water.

8.8.5 Place phosphoric acid treated glass wool into bottom end of Pasteur pipette.

8.8.6 Fill Pasteur pipettes with 1 mL of cation-exchange resin. They are ready to use when excess fluid drains from column.

9. Apparatus Preparation for Method A

9.1 *Condition gas chromatograph columns*—Prior to experimentation, all GC columns must be conditioned for use. Conditioning activates the stationary phase removing contaminants that reduce the number of active sites that interfere with analysis. This conditioning requires heating the column for an extended period of time with the carrier gas flowing through it, (refer to the operator's manual of the GC for apparatus-specific instructions).

9.1.1 Detach the column (effluent end) from the detector. Allow carrier gas to flow through column, but not to pass into detector. Heat the oven to the manufacture's recommended conditioning temperature. This is usually 50 to 60°C higher than operating temperature. If the temperature is too high it could breakdown the stationary phase. Leave the column conditioning overnight, or at least for 4 h. Refer to Table 05.10-A2 for details.

Table 05.10-A2 Parameters for gas chromatograph column conditioning.

Stationary Phase	*Oven Temp (°C)*	*Carrier Gas*	*Flow Rate (mL min^{-1})*
5% neopentyl glycol sebacte plus 1% H_3PO_4 on Anakrom Polyester	165	OFN	20
Chromosorb 101	250	N_2	20
SP 1200	175	N_2	20
Carbowax	175	N_2	20
Nukol	165	H_2	20

9.2 *Pretreat columns*—After conditioning, columns are sometimes pretreated before use.

9.2.1 *Chromosorb 101*—Inject 5 µL of water into the column 20 times; SP1200 inject 1 µL of water into the column 10 times.

9.2.2 *Carbowax*—With the injection port held at 175°C inject 5 µL of formic acid (0.1%) into the column 7 times.

9.2.3 *Anakrom Polyester* —Inject 10% formic acid onto the column. If resolution deteriorates during analysis perform this pretreatment procedure between samples.

9.3 *Identification of VFAs:*

9.3.1 One µL of each standard solution is injected into the gas chromatograph and the chromatogram is analyzed. In most chromatograms there is a solvent peak eluted close to zero time and is followed by a VFA peak corresponding to the standard acid under test. The peak retention time of the VFA peak is measured from the graph. This is the time it takes the acid to reach its maximum desorption rate, refer to Fig 05.10-A2 This time depends upon the gas chromatographic conditions used and any change in the conditions is very likely to change the peak retention time. This peak retention time is used as a marker to identify VFAs in the aqueous samples of the compost.

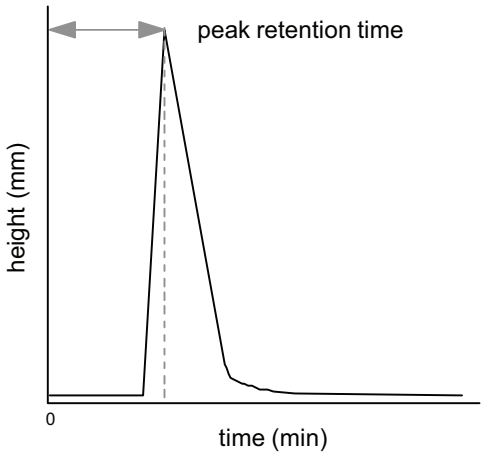

Fig 05.10-A2 Peak retention time illustration.

9.3.2 One µL of mixed standard solution is injected into the gas chromatograph and the chromatogram is analyzed. The peak retention times of the VFAs are measured and used to identify VFAs in the aqueous sample of the compost. Low concentrations of acids might be found in the traces, (e.g., methyl valeric acid). Large concentrations of phenols can interfere with the n-caproic acid peak.

9.3.3 The VFAs should elute in the following ascending order: acetic acid, propionic acid, isobutyric acid, n-butyric acid, isovaleric acid, n-valeric acid, isocaproic acid, n-caproic acid and n-heptanoic acid. The peak retention is lowest for acetic and highest for n-heptanoic acid.

9.4 Sensitivity of the column is determined by establishing the lowest concentration (moles L^{-1}) that each VFA (C_2 through C_7) is detectable by GC.

9.4.1 Dilute the mixed standard solution two-fold and inject 1 µL of sample into the GC. A two-fold dilution of the 10 m*M* standard solution is 2.5 m*M*.

9.4.2 Repeat 9.4.1 until no peak is detected. The sensitivity of the column to a particular VFA is the lowest concentration (mmoles L^{-1}) detectable.

10. Procedure for Method A

10.1 Place 5.00 g of compost at as-received moisture into 100-mL conical flasks and add 50 mL of phosphate buffer (0.2 mol L^{1}) at pH 7.0. Label this bottle as *Compost sample*. Place 50 mL of phosphate buffer (0.2 mol L^{-1}) into conical flask and label this flask *Control*. Seal the bottles with permeable caps and incubate and agitate at room temperature (20-24°C).

10.1.1 Determine moisture content of parallel aliquot of compost. Oven dry a 25 g sample aliquot at 70±5°C until weigh change diminishes to nil, approximately 2 d.

10.2 After 30 min, remove 3 mL of solution with a disposable plastic syringe from the flask labeled *Compost sample*.

10.3 Place 2.7 mL of this compost solution into a glass vial and add 0.3 mL of 0.1% formic acid. Store acidified sample in freezer at -10°C.

10.4 Repeat steps 10.2 and 10.3 for the *Control sample*.

10.5 *Sample Pretreatment*

NOTE 2A—Pretreatment might not be necessary if there are no significant interference from phenols, etc., or when the solution is very clear.

10.5.1 *Vacuum Distillation:*

10.5.1.1 Place 2 mL of sample into round- bottomed specimen flask and place it into oil bath at ambient laboratory temperature (Fig 05.10-A1). Cool the receiver tube with liquid nitrogen and start the suction pump. Evacuate the system to approximately 5 torr and hold this pressure by continuous suction.

10.5.1.2 Under continuous suction, the contents of the specimen flask are mixed with a magnetic stirrer and slowly heated from ambient laboratory temperature to 120°C over a time period of 15 to 20 min. After this time period the specimen should be completely dry.

10.5.1.3 The distillate collected in the receiver tube is thawed at room temperature and stored in glass vials for immediate GC analysis.

10.5.2 *Molecular Sieving*—This pretreatment is not appropriate for viscous samples.

10.5.2.1 Place 1 mL of acidified sample onto the cation exchange column and collect effluent flowing through the column.

10.5.2.2 Rinse column three times with 0.5 mL of 0.1 % formic acid and collect effluent. Combine effluent in a glass vial and store at -10°C for GC analysis.

10.6 *GC Standards*—After the column is conditioned (step 9.1), pretreated (step 9.2) and the peak retention times of the standard VFAs tabulated (step 9.3) calibration curves are performed.

10.6.1 Inject 1 µL of the lowest concentration standard (0.09 mmoles L^{1}) into the GC and determine peak height. Refer to Table 05.10-A1 for conditions to operate GC.

10.6.2 Repeat step 10.6.1 for all the standards (0.9, 4.5 and 9 mmoles L^{-1}, from lowest to highest concentration of VFA.

10.6.3 Determine the calibration curves for each VFA. Plot the peak height (y-axis, dependent variable) versus the mmol L^{1} of VFA in solution (x-axis, independent variable). Fit a linear calibration line.

10.7 *Perform GC Analysis of Samples:*

10.7.1 Inject 1 µL of a sample into the GC and determine the peak height. After each injection the syringe is washed with water, ethanol and ethyl ether and dried by suction. Refer to Table 05.10-A1 for conditions to operate GC.

10.7.2 Periodically (every 20 samples or less) test the status of the column and inject standard samples into the column. If the resolution deteriorates the column might require to be pretreated (step 9.2).

10.7.3 Determine the concentration of each VFA in the sample.

11. Calculations for Method A

11.1 *Linear Calibration Curve:*

$$H = M \times C + B \qquad \textit{Equation 11.1}$$

where:

H = the peak height (mm) for the standard samples,
C = concentration of each VFA (mmol L^{-1}) in the standard samples,
M = slope of fitted curve (mm/mmol L^{-1}), and
B = intercept of fitted curve (mm).

11.2 *Determine VFA*—Calculate the concentration of each VFA injected into the GC by use of the above calibration curve (Equation 11.1) where H and C are now the peak height (mm) and concentration of each VFA (mmol L^{-1}) in the compost sample, respectively:

$$C = (H - B) \div M \qquad \textit{Equation 11.2}$$

11.3 Calculate the mmoles of each VFA in the flask:

11.3.1 No pretreatment of sample or sample pretreated by vacuum distillation:

$$E = C \times 1.111 \times 0.050\ L \qquad \textit{Equation 11.3.1}$$

11.3.2 Sample pretreated by molecular sieving:

$$E = C \times (2.5 \times 1.111) \times 0.050\ L \qquad \textit{Equation 11.3.2}$$

11.4 Calculate mmoles of each VFA per g of oven-dried compost:

$$F = E \div (5 \times R) \qquad \textit{Equation 11.4}$$

11.5 Calculate the mmoles of total VFA per g of oven dried compost in each sample:

$$G = \Sigma F \qquad \textit{Equation 11.5}$$

where:

H = peak height, mm,
M = slope of fitted curve, mm $(\text{mmol } L^{-1})^{-1}$,
C = concentration of each VFA, mmol L^{-1},
B = intercept of fitted curve, mm,
E = mmoles of each VFA in flask,
1.111 = dilution factor, (3.0 mL ÷ 2.7 mL),
5 = mass of compost sample, g as-received moist basis,
2.5 = dilution factor, (2.5 mL ÷ 1 mL),
F = mmoles of each VFA per g of compost,
G = total VFA, mmoles g^{-1} of compost, and
R = ratio of oven dried sample weight versus as-received weight, unitless.

05.10 METHODS SUMMARY

12. Report

12.1 *Method 05.10-A Volatile Fatty Acids in Compost Extract by Gas Chromatography*—Report mmoles of each VFA per g dw of compost and mmoles of Total VFAs per g dw of compost. Report sample moisture, %, wet weight basis.

13. Precision and Bias

13.1 *Method 05.10-A Volatile Fatty Acids in Compost Extract by Gas Chromatography*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

14. Keywords

14.1 vacuum distillation; gas chromatography; volatile fatty acids; short chain fatty acids

06.00 ANALYSIS OF SYNTHETIC ORGANIC CHEMICALS IN COMPOST

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Introduction

1.1 This section presents some key points of compost sample collection, handling and analysis for a wide range of synthetic organic compounds. Recommendations for proper handling and analysis of waste materials and soils are generally available. Important characteristics of mature compost versus raw waste or soil that dictate handling practices and requirements are presented.

1.2 Biological activity and microbial populations of compost are substantially higher than activity and populations in most other materials. As a result, rapid biodegradation or biotransformation of synthetic organic compounds in the compost may occur if the samples are not handled properly. Second, compost is a chemically active material, with the ability to adsorb many organic compounds and, in some cases, to promote chemical degradation.

1.3 Enclosing samples in sealed containers for shipment to an analytical laboratory is likely to change the compost from a prevailingly aerobic material to an anaerobic material. This change can promote biological and/or chemical changes in synthetic organic compounds that would not occur under aerobic conditions. A key feature in minimizing such changes is to reduce the temperature of collected samples to 4°C as rapidly as possible and to maintain the samples at this temperature until extraction or analysis is performed.

2. Sample Collection and Initial Handling

2.1 Sample collection and preservation is described in TMECC 02.01. A list of storage containers appropriate for use with organic contaminant samples are presented in Table 06.00-1.

2.2 In advance of collecting samples, have available a container of ice or a refrigerator into which the samples can be placed immediately after collection. All samples must be collected in glass jars with inert liners, (e.g., Teflon). The customary practice of using plastic bags or jars with liners of ordinary materials such as plastic or cardboard is inappropriate when organic analyses will be performed, because many analytes are absorbed by plastic and cardboard. Any absorption that occurs during transport and storage effectively reduces the amount of analyte present in the actual sample by the time the analysis is conducted. This can bias results.

2.3 Fill the jars to the top and pack the material as firmly as possible to minimize air space (into which volatile contaminants can move and be lost when the jar is opened).

2.4 Immediately after collection, put the jars in a refrigerator or ice with adequate space between them to allow rapid heat dissipation.

3. Packaging and Shipping to Analytical Laboratory

3.1 After the samples are well-cooled, seal each glass vial in a separate small plastic container, (e.g., Ziploc® storage bag).

3.2 Wrap the storage bag around the bottle to minimize air space, then close the zipper and wrap the bag with packing tape.

3.3 Wrap each vial and bag in small size bubble wrap and secure the wrap with tape. If there is not enough space in the shipping container to wrap each vial separately in bubble wrap, sandwich and arrange the containers in the shipping box as follows: vial in bubble wrap – vial in bag – vial in bubble wrap.

3.4 Put doubled plastic bags of a size to fill the container in the shipping carton. Shipping containers can be either hard plastic or polystyrene coolers placed inside of a cardboard box. Pack the space between jars with ice in plastic bags or chemical ice packs.

3.5 Fill the extra volume of the containers with bubble-wrap to minimize shifting of contents.

3.6 Include a *chain of custody form* with analytical instructions in a plastic bag at the top of the contents inside of the shipping container. Refer to the chain of custody example form presented in Fig 02.01-E1 Chain of Custody.

3.7 Secure the label and lid of container by wrapping with nylon reinforced tape all the way around the

container. Put the tape also over the edges of the shipping label. Labels sometimes do not adhere well to the hard shell coolers.

3.8 Send the containers by overnight express early in the week. Do not ship on Thursday or Friday, because packages may be stored under severe conditions (hot or cold) over the weekend. Changes in the samples can occur under such conditions.

Table 06.00-1 *Synthetic Organic Compounds:* Sampling containers and conditions for compost and source ingredient testing.

Test Parameter of Interest	*Container*	*Conditions*	*Maximum Holding Time Allowed in Lab*
Chlorinated Herbicides	G, Teflon lined cap (2-$^1/_2$ L.A.J.)	4°C	7 d until extraction
Chlorinated Hydrocarbons	G, Teflon lined cap (2-$^1/_2$ L.A.J.)	4°C	7 d until extraction
Chlorinated Pesticides	16 oz B.R. (2-$^1/_2$ L.A.J.)	4°C	7 d until extraction
Dioxins & Furans	G, Teflon lined cap (2-$^1/_2$ L.A.J.)	4°C store in dark	7 d until extraction
Nitroaromatics and isophorone	G, Teflon-lined cap (2-$^1/_2$ L.A.J.)	4°C store in dark	7 d until extraction
PCB	G, Teflon lined cap (2-$^1/_2$ L.A.J.)	4°C	7 d until extraction
Phthalate esters	G, Teflon lined cap	4°C	7 d until extraction
Polycyclic Aromatic Hydrocarbons (PAH)	G, Teflon-lined cap (2-$^1/_2$ L.A.J.)	4°C store in dark	7 d until extraction
Purgeable aromatic hydrocarbons	G, Teflon lined septum (40-mL Glass V)	4°C	14 d prior lab testing
Semi-Volatile Organics	G, Teflon-lined Septum (2.5-L Jug)	4°C	7 d
TCLP Sample	G, Teflon-lined Septum (2.5-L Jug)	4°C	7 d until extraction
Volatile Organic Compounds (VOC)	G, Teflon lined septum (40-mL Glass V)	4°C	14 d preserved in HCl†

NOTE 1—P=Plastic; G=Glass, HDPE=High Density Polyethylene
†—Evaluation data is being sought to confirm this requirement for curing and finished composts.

4. Sample Handling and Storage at Laboratory

4.1 Maintain samples at 4°C during the entire time before samples are extracted or analyzed to avoid compositional changes due to biological activity or chemical reactions.

5. Extraction and Analysis of Samples

5.1 *Analysis of Specific Compounds*—A few procedures for analysis of specific compounds in compost have been published, but, unless the documents demonstrate that the procedures give good recovery of analytes, their use is not recommended. Procedures developed for analysis of soil samples may not be adequate for compost analysis because of the differences in chemical composition of soil and compost. The majority of soils contain less than 6% organic matter, while mature compost typically has about 30% organic matter. For hydrophobic organic chemicals such as chlorinated pesticides, PCBs, and polynuclear aromatic compounds, adsorption to organic matter is often strong, which can affect extractability of contaminants. With the high organic content of compost, quantitative desorption can be difficult to achieve. In addition, the mineralogy of compost has not been studied in sufficient detail to know the mineral composition of the compost. Hence, mineral-enhanced chemical degradation of analytes (particularly at the elevated temperatures used for some extraction procedures) during the process must be considered. Iron-containing minerals are particularly active in these processes; these minerals make up 2% to 3% of the dry weight of mature compost. Formation of insoluble dark brown precipitates during solvent evaporation (e.g., after Soxhlet extraction) is a common phenomenon with compost extracts. Such precipitation is indicative of chemical polymerization reactions, but the reactants (perhaps analytes in some cases) have not been characterized.

5.1.1 *Recommendation*—When well-documented analytical procedures are not available for the analyte in question, please refer to US EPA procedures from *SW-846, Methods for Analysis of Solid Wastes*. Complete procedures are available on-line at: *http://www.epa.gov/epaoswer/hazwaste/test/main.htm*.

5.1.2 Appropriate procedures for analysis of petroleum hydrocarbons are given at the US EPA Underground Storage Tank Program web site:

http://www.epa.gov/swerust1/cat/samb-1.htm.

5.2 *Immunoassay Procedures*—There is little available experience on the use of immunoassay procedures for analysis of compost. These are attractive procedures because they can be performed on-site and they are rapid and inexpensive in comparison to sending samples to a commercial laboratory. Since aqueous extracts of compost will contain humic materials that bind avidly to proteins (and thereby may interfere with antigen–antibody reactions of immunological procedures), verification of data validity should be done before widespread adoption of these procedures for compost analysis.

5.2.1 *Recommendation*—Until sufficient experience and confidence in procedures is gained by a laboratory, addition of known compounds to compost prior to extraction and analysis should be standard practice so that the laboratory and the client can have confidence in the data.

5.3 Mature compost contains water- and solvent-soluble low molecular weight compounds of many types; immature composts probably have an even wider variety of solubles. These compounds extract readily with the solvents that are widely used for standard procedures (e.g., methanol, ethyl acetate, acetone, methylene chloride). All of these compounds can interfere with identification of contaminants when samples are analyzed by gas chromatography (GC) or high-pressure liquid chromatography (HPLC), since they may elute from a column with the same retention time as a target analyte. If analysis were done only by GC with a relatively non-specific detector such as a flame ionization detector (FID), there is a high probability that some potential analytes of concern would have identical retention times to these naturally-occurring compounds and the erroneous conclusion would be that the compost was contaminated. In some cases, natural products in the compost are also compounds of environmental concern (e.g., some degradation products of lignin and phenolic constituents of plant tissues are listed in US EPA phenols analysis according to Method 8040A). When compost that is not likely to be contaminated with hydrocarbons is analyzed by extraction and GC-FID, an apparent total petroleum hydrocarbons (TPH) content of about 100 mg kg^{-1} is often found. In fact, the TPH value is likely to be other types of chemicals that are naturally found in composting materials.

5.3.1 *Recommendation*—Because of the potential for misidentification of compounds by GC and HPLC, use of GC-MS analysis is recommended whenever possible. If GC-MS analysis is not feasible, use of detectors that are most selective is recommended (e.g., electron capture for halogenated compounds or nitrogen-phosphorus for many common pesticides). It is recognized that this recommendation is a more rigorous analytical approach than given in US EPA's SW-846, in which many procedures rely solely upon GC-FID or HPLC methods.

Test Method: Chlorinated Herbicides							**Units:** mg kg^{-1} or µg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
06.01							06.01	

06.01 CHLORINATED HERBICIDES

1. Significance

1.1 Residues of chlorinated herbicides can sometimes be found in compost. A properly managed composting process will result in the breakdown of this material and render it harmless.

2. Selection of Method

2.1 *Sample Preparation:*

2.1.1 *US EPA Method 8151A*—Chlorinated Herbicides by Gas Chromatography.

2.2 *Sample Analysis:*

2.2.1 *US EPA Method 8151A*—Chlorinated Herbicides by GC Using Methylation or Pentafluorobenzylation Derivatization.

Table 06.01-1 Chlorinated herbicides by gas chromatography

Compound	*Chemical Abstract Service Registry Number*
2,4-D	94-75-7
2,4-DB	94-82-6
2,4,5-TP (Silvex)	93-72-1
2,4,5-T	93-76-5
Dalapon	75-99-0
Dicamba	1918-00-9
Dichloroprop	120-36-5
Dinoseb	88-85-7
MCPA	94-74-6
MCPP	93-65-2
4-Nitrophenol	100-02-1
Pentachlorophenol	87-86-5
Acifluorfen	50594-66-6
Bentazon	25057-89-0
Chloramben	133-90-4
DCPA diacid*	2136-79-0
3,5-Dichlorobenzoic acid	51-36-5
5-Hydroxydicamba	7600-50-2
Picloram	1918-02-1

* DCPA monoacid and diacid metabolites included in method scope; DCPA diacid metabolite used for validation studies. DCPA is a dimethyl ester.

ADAPTED FROM—SW-846 US EPA Method 8151A. Chlorinated Herbicides by GC.

Test Method: Dioxin/Furans							**Units:** mg or µg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
06.02							06.02	

06.02 DIOXIN/FURANS

1. Significance

1.1 Dioxin was found at low levels in some paper manufactured from wood pulp bleached with chlorine gas, so the paper industry changed its bleaching procedure to avoid creation of dioxin/furans. The dioxins/furans in the environment are distributed by atmospheric deposition onto organic materials such as leaves and grass that become feedstocks for composting. Dioxin is carcinogenic to some animals.

2. Selection of Method

2.1 *Sample Preparation:*

2.1.1 *US EPA Method 8280A*—Extraction and Cleanup Procedures. Follow the procedure outline for Soil Samples.

2.2 *Sample Cleanup:*

2.2.1 *US EPA Method 8280A*—Carbon Column Cleanup.

2.3 *Sample Analysis:*

2.3.1 *US EPA Method 8280A*—The Analysis of Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofurans. This method is appropriate for the determination of tetra-, penta-, hexa-, hepta-, and octachlorinated dibenzo-*p* dioxins (PCDDs) and dibenzo furans (PCDFs) This method uses high-resolution capillary column gas chromatography/low resolution mass spectrometry (HRGC/LRMS). The resolution is 2-10 ppb.

2.3.2 *US EPA Method 8290*—The Analysis of Polychlorinated Dibenzo-*p*-Dioxins and Polychlorinated Dibenzofurans. This analytical method calls for the use of high resolution gas chromatography, gas chromatography and high resolution mass spectrometry (HRGC/HRMS) on purified sample extracts.

Test Method: Organochlorine Pesticides						**Units:** mg or µg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							06.03	

06.03 ORGANOCHLORINE PESTICIDES

REFER TO METHOD 06.05 FOR CLEANUP GUIDELINES.

1. Significance

1.1 Residues of organochlorine pesticides are sometimes found in compost. A properly managed composting process will result in the breakdown of this material, and render it harmless. The only exception to this is DDT.

2. Selection of Method

2.1 *Sample Preparation:*

2.1.1 *US EPA Method 3540C*—Soxhlet Extraction for volatiles, non-volatiles, and semivolatiles.

2.2 *Sample Cleanup:*

2.2.1 *US EPA Method 3610B*—Alumina Cleanup or US EPA Method 3620B—Florisil Cleanup. To eliminate the material of higher boiling point that could plug-up injection ports it might be necessary to use US EPA Method 3640—Gel-Permeation Cleanup and US EPA Method 3660—Sulfur Cleanup, if the sample contains elemental sulfur.

2.3 *Sample Analysis:*

2.3.1 *US EPA Method 8081A*—Organochlorine Pesticides, Halowaxes and PCBs as Aroclors by Gas Chromatography: Capilliary Column Technique.

Table 06.03-1 Organochlorine Pesticides and Polychlorinated Biphenyls by Gas Chromatography.

Compound Name	*Chemical Abstract Services (CAS) Registry Number*	*Compound Name*	*Chemical Abstract Services (CAS) Registry Number*
Alachlor	15972-60-8	Endosulfan I	959-98-8
Aldrin	309-00-2	Endosulfan II	33213-65-9
Aroclor-1016 (PCB)	12674-11-2	Endosulfan sulfate	1031-07-8
Aroclor-1221 (PCB)	1104-28-2	Endrin	72-20-8
Aroclor-1232 (PCB)	11141-16-5	Endrin aldehyde	7421-93-4
Aroclor-1242 (PCB)	53469-21-9	Endrin ketone	53494-70-5
Aroclor-1248 (PCB)	12672-29-6	Etridiazole	2593-15-9
Aroclor-1254 (PCB)	11097-69-1	Halowax-1000	58718-66-4
Aroclor-1260 (PCB)	11096-82-5	Halowax-1001	58718-67-5
α-BHC	319-84-6	Halowax-1013	12616-35-2
β-BHC	319-85-7	Halowax-1014	12616-36-3
δ-BHC	319-86-8	Halowax-1051	2234-13-1
γ-BHC (Lindane)	58-89-9	Halowax-1099	39450-05-0
Captafol	2425-06-1	Heptachlor	76-44-8
Captan	133-06-2	Heptachlor epoxide	1024-57-3
Chlorobenzilate	510-15-6	hexachlorobenzene	118-74-1
α-Chlordane	5103-71-9	Hexachlorocyclopentadiene	77-47-4
γ -Chlordane	5103-74-2	Isodrin	465-73-6
Chloroneb	2675-77-6	Kepone	143-50-0
Chloropropylate	99516-95-7	4,4’-Methoxychlor	72-43-5
Chlorothalonil	1897-45-6	Mirex	2385-85-5
DBCP	96-12-8	Nitrofen	1836-75-5
DCPA	1861-32-1	PCNB	82-68-8
4,4’-DDD	72-54-8	Perthane	72-56-0
4,4’-DDE	72-55-9	Propachlor	1918-16-17
4,4’-DDT	50-29-3	Strobane	8001-50-1
Diallate	2303-16-4	Toxaphene	8001-50-2
Dichlone	117-80-6	Trans-Nonachlor	39765-80-5
Dicofol	115-32-2	trans-Permethrin	51877-74-8
Dieldrin	60-57-1	Trifluralin	1582-09-08

ADAPTED FROM—SW-846 US EPA Method 8081A-1. Organochlorine Pesticides, Halowaxes and PCBs as Aroclors by Gas Chromatography: Capilliary Column Technique.

Test Method: Organophosphorus Pesticides						**Units:** mg or µg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							06.04	

06.04 ORGANOPHOSPHORUS PESTICIDES

1. Significance

1.1 Residues of organophosphorus (OP) pesticides can be found in compost (Refer to *Table 06.04-1* for list of potential OP contaminants). A properly managed composting process will result in the breakdown of this material, and render it harmless.

2. Selection of Method

2.1 *Sample Preparation:*

2.1.1 *US EPA Method 3540C*—Soxhlet Extraction for volatiles, non-volatiles, and semivolatiles.

2.1.2 *US EPA Method 3550B*—Ultrasonic Extraction for non-volatiles and semivolatiles.

2.2 *Sample Cleanup:*

2.2.1 *US EPA Method 3620B*—Florisil Cleanup and if the sample contains elemental sulfur then use US EPA Method 3660B—Sulfur Cleanup.

2.3 *Sample Analysis:*

2.3.1 *US EPA Method 8141A*—Organophosphorus Pesticides by Gas Chromatography Capillary Column Technique.

Table 06.04-1 Organophosphorus pesticides by gas chromatography, capillary technique.

Compound Name	*Chemical Abstract Services (CAS) Registry Number*	*Compound Name*	*Chemical Abstract Services (CAS) Registry Number*
Azinphos-methyl	86-50-0	Fensulfothion	115-90-2
Azinphos-ethyl	2642-71-9	Fonophos	944-22-9
Bolstar (Sulprofos)	35400-43-2	Fenthion	55-38-9
Carbophenothion	786-19-6	Leptophos	21609-90-5
Chlorofenvinphos	470-90-6	Malathion	121-75-5
Chlorpyrifos	2921-88-2	Merphos	150-50-5
Chlorpyrifos methyl	5598-13-0	Mevinphos	7786-34-7
Coumaphos	56-72-4	Monocrotophos	6923-22-4
Crotoxyphos	7700-17-6	Naled	300-76-5
Dementon-0	8065-48-3	Parathion , ethyl	56-38-2
Dementon-S	8065-48-3	Parathion, methyl	298-00-0
Diazinon	333-41-5	Phorate	298-02-2
Dichlorofenthion	97-17-6	Phosmet	732-11-6
Dichlorvos	62-73-7	Phosphamidon	13171-21-6
Dicrotophos	141-66-2	Ronnel	299-84-3
Dimethoate	60-51-5	Stirophos	22248-79-9
Dioxathion	78-34-2	Sulfotepp	3689-24-5
Disulfoton	298-04-4	TEPP	21646-99-1
EPN	2104-64-5	Terbufos	13071-79-9
Ethion	563-12-2	Thionazin	297-97-2
Ethoprop	13194-48-4	Tokuthion	34643-46-4
Famphur	52-85-7	Trichlorfon	52-68-6
Fenithrothion	122-14-5	Trichloronate	327-98-0

ADAPTED FROM—SW-846 US EPA 8141A-1. Organophosphorus Pesticides by Gas Chromatography Capillary Column Technique.

Test Method: Polychlorinated Biphenyls						**Units:** mg or µg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							06.05	

06.05 POLYCHLORINATED BIPHENYLS

CAUTION—To avoid significant systematic errors, carefully review and follow the observations discussed in this section and carefully follow the referenced US EPA methods for evaluating PCBs in solid waste.

1. Significance

1.1 Polychlorinated biphenyls (PCBs) are a family of industrial compounds produced by chlorination of biphenyl. It is an environmental pollutant that accumulates in animal tissue with resultant pathogenic and carcinogenic effects, and can cause malformations of embryos or fetus.

1.2 Mixtures of PCBs of various chlorine compounds were sold for many years in the US by Monsanto Co. under the tradename Aroclor (1200 series and 1016), Table 06.05-1. The Aroclors are no longer marketed, but are sometimes found in the environment. The Aroclors most commonly found are 1242, 1254, and 1260. PCBs were once a component of carbonless copy paper and found in waste streams. Manufacture and use of PCBs in carbonless copy paper was discontinued June 1, 1971. Concentrations are generally very low, they are usually not found in MSW feedstock or in yard waste feedstocks.

2. Selection of Method

2.1 *Sample Extraction and Preparation:*

2.1.1 *US EPA Method 3540C*—Soxhlet Extraction for volatiles, non-volatiles, and semivolatiles.

2.2 *Sample Cleanup*—Use the following methods according to EPA guidelines.

2.2.1 *US EPA Method 3665A*—Sulfuric Acid/Permanganate followed by one of the following procedures:

2.2.1.1 *US EPA Method 3620B*—Florisil,

2.2.1.2 *US EPA Method 3630C*—Silica-Gel, or

2.2.1.3 *US EPA Method 3610B*—Alumina Cleanup.

2.2.2 If the sample contains elemental sulfur it will interfere with the gas chromatographic detectors and it may be necessary to use US EPA Method 3660—Sulfur Cleanup. However, sulfur will generally elute in the first fraction of the Florisil cleanup, so this cleanup should be sufficient to remove most of the elemental sulfur.

2.2.3 The silica-gel cleanup provides the best separation of PCBs from most single component organochlorine pesticides. If only PCBs are to be measured, this method should be used in conjunction with the sulfuric acid/permanganate cleanup.

2.2.4 A chemically inert compound not expected to occur in the sample should be added to each sample and blank. Common surrogate standards for PCBs are 2,4,5,6 tetrachloro-meta-xylene (TCMX) and dibutylchlorendate (DBC). They should be added at a concentration of 5 mg mL^{-1} to compost. They are used to check the extraction efficiency for the individual sample and blanks. If the recovery rate varies corrective action must be performed. For example, check for contamination in the reagents and cleanliness of the glassware and possible contamination form plastic. The sample containers used to collect samples for the determination of PCBs should be made of glass or Teflon, and have screw caps with Teflon lined septa. Plastic containers should not be used due to the possibility of contamination from phthalate esters and other hydrocarbons from the plastic.

2.3 *Sample Analysis:*

2.3.1 *US EPA Method 8081A*—Organochlorine Pesticides by Gas Chromatography: Capilliary Column Technique.

2.3.1.1 Capillary columns must be used rather than packed columns. Fused silica, open-tubular columns offer improved resolution, better selectivity, increased sensitivity and faster analysis. In addition, capillary columns are better for detecting the congeners of PCBs. Congeners are stable components that make up the various Aroclors or PCBs, for example, biphenyl, 2-chloro-biphenyl, and 2,3-dichlorobiphenyl.

2.3.1.2 PCBs are composed of numerous compounds, which generate multi-peak chromatograms. Also, the chromatogram of the residue may not match that of the standard.

2.3.1.3 Use fresh calibration standards. A mixture of Aroclors 1016 and 1260 will suffice for the initial

calibration curve of all Arocolors since they include all congeners present in the different regulated PCBs. A midpoint calibration of each Aroclors must be included with the initial calibration to determine the Arocolor pattern and retention time on each column.

2.3.1.4 To quantitate PCBs, compare the total area or height of peaks of the chlorinated biphenyl peaks to the total area or height of peaks from the appropriate PCB reference material. Measure the total area or height response from common baseline under all peaks. Use only those peaks that can be attributed to chlorobiphenyls. Always quantitate peak-to-peak. Mixtures of Aroclors may be required to provide the best match of GC patterns of sample and reference material. See *US EPA SW-846 Method 8081A-17*.

2.3.1.5 The analysis of PCBs requires the temperature of the oven housing the capillary column to be ramped slowly from an initial temperature to a final temperature. A lower temperature is required to allow the low molecular weight PCBs to desorb from the column without interference from the solvent front and the low molecular weight congeners. The slow ramping ($10°C\ min^{-1}$) and higher temperature enables the higher molecular weight PCBs to desorb from the column.

Table 06.05-1 Polychlorinated biphenyls (PCBs).

Compound	*Chemical Abstract Service Registry Number*	*Compound*	*Chemical Abstract Service Registry Number*
Aldrin	309-00-2	Alachlor	15972-60-8
α-BHC	319-84-6	Captafol	2425-06-1
β-BHC	319-85-7	Chloroneb	2675-77-6
γ-BHC (Lindane)	58-89-9	Chloropropylate	99516-95-7
δ-BHC	319-86-8	Chlorothalonil	1897-45-6
Chlorobenzilate	510-15-6	DCPA	1861-32-1
α-Chlordane	5103-71-9	Dichlone	117-80-6
γ-Chlordane	5103-74-2	Dicofol	115-32-2
Chlordane - not otherwise specified	57-74-9	Etridiazole	2593-15-9
DBCP	96-12-8	Halowax-1000	58718-66-4
4,4'-DDD	72-54-8	Halowax-1001	58718-67-5
4,4'-DDE	72-55-9	Halowax-1013	12616-35-2
4,4'-DDT	50-29-3	Halowax-1014	12616-36-3
Diallate	2303-16-4	Halowax-1051	2234-13-1
Dieldrin	60-57-1	Halowax-1099	39450-05-0
Endosulfan I	959-98-8	Mirex	2385-85-5
Endosulfan II	33213-65-9	Nitrofen	1836-75-5
Endosulfan sulfate	1031-07-8	PCNB	82-68-8
Endrin	72-20-8	Permethrin	51877-74-8
Endrin aldehyde	7421-93-4	Perthane	72-56-0
Endrin ketone	53494-70-5	Propachlor	1918-16-7
Heptachlor	76-44-8	Strobane	8001-50-1
Heptachlor epoxide	1024-57-3	*trans*-Nonachlor	39765-80-5
Hexachlorobenzene	118-74-1	Trifluralin	1582-09-8
Hexachlorocyclopentadiene	77-47-4		
Isodrin	465-73-6		
Methoxychlor	72-43-5		
Toxaphene	8001-35-2		

ADAPTED FROM—SW-846 US EPA Method 8081A. Organochlorine Pesticides by Gas Chromatography.

3. Referenced Documents

Environmental SFE of Polychlorinated biphenyls (PCBs) in Solid Waste. Hewlett Packard Application Note 228-282. June 1994.

Municipal Solid Waste Compost Utilization Program. Volume I: Executive Summary. MN OEA. February 29, 1996.

Test Methods for Evaluating Solid Waste. Volume 1B. Laboratory Manual Physical/Chemical Methods. US EPA SW-846.

Test Method: Semivolatile Organic Compounds							**Units:** mg or µg kg^{-1} dw	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							06.06	

06.06 SEMIVOLATILE ORGANIC COMPOUNDS

1. Scope

1.1 Semivolatile organic compounds have higher vapor pressures and boiling points than volatile organic compounds. This test method screens for a broad category of organic compounds that can be measured by GC/MS without derivatization. Test Methods for Evaluating Solid Wastes, SW-846 US EPA Method 8270B lists more than 250 semivolatile organic compounds.

2. Selection of Method

2.1 *Sample Preparation:*

2.1.1 *US EPA Method 3540C*—Soxhlet Extraction, or

2.1.2 *US EPA Method 3541A*—Automated Soxhlet Extraction

2.2 *Sample Cleanup:*

2.2.1 *US EPA Method 3610B*—Alumina Cleanup or

2.2.2 *US EPA Method 3620B*—Florisil Cleanup.

2.2.3 *US EPA Method 3640A*—Gel-Permeation Cleanup to eliminate the material of higher boiling point that plug injection ports.

2.3 *Sample Analysis:*

2.3.1 *US EPA Method 8270C*—Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS): Capillary Column Technique. This method can be used to quantify most neutral, acidic and basic organic compounds that are soluble in methylene chloride and capable of being eluted without derivatization as sharp peaks from a gas chromatographic column. Examples of compounds include polynuclear aromatic hydrocarbons, chlorinated hydrocarbons and pesticides, phthalate esters, organophosphate esters, nitrosamines, haloethers and aldehydes. Refer to Table 1 in *SW-846 US EPA Method 8270B* for the list of compounds analyzed.

Test Method: Volatile Organic Compounds						**Units:** mg or µg kg^{-1} dw		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							06.07	

06.07 VOLATILE ORGANIC COMPOUNDS

1. Scope

1.1 Volatile organic compounds are low molecular weight molecules and have low boiling points and vapor pressures. They include aliphatics (e.g., acetone), aromatics (e.g., benzene) and halogen containing organic compounds (e.g., carbon tetrachloride). They are recognized for their distinctive odors.

2. Selection of Method

2.1 *Sample Preparation:*

2.1.1 *US EPA Method 3540C*—Soxhlet Extraction, or

2.1.2 *US EPA Method 3541A*—Automated Soxhlet Extraction

2.2 *Sample Analysis:*

2.2.1 *US EPA Method 8011*—1, 2-Dibromoethane and 1, 2-Dibromo-3-chloropropane by Gas Chromatography. These compounds have been tentatively classified as known or suspected human or mammalian carcinogens. This procedure was derived for drinking and ground water and is not tested for compost or waste solids.

2.2.2 *US EPA Method 8015B*—Nonhalogenated Volatile Organics by Gas Chromatography. This method is suitable for measuring acrylamide, diethyl ether, ethanol, methyl ethyl ketone, methyl isobutyl ketone and paraldehyde. Samples may be analyzed by direct injection from the headspace of a compost sample or via purge-and-trap refer to method US EPA 5030.

2.2.3 *US EPA Method 8021A*—Aromatic and Halogenated Volatiles by Gas Chromatography Using Photoionization and/or Electrolytic Conductivity Detectors. This method is used to determine the concentration of various aromatic volatile organic compounds that include benzene, chlorobenzene, 1,2 dichlorobenzene, 1,3 dichlorobenzene 1,4 dichlorobenzene, ethylbenzene, toluene and xylenes. Samples may be analyzed by direct injection from the headspace of a compost sample or via purge-and-trap refer to method US EPA 5030.

2.2.4 *US EPA Method 8031*—Acrylonitrile by Gas Chromatography

2.2.5 *US EPA Method 8032A*—Acrylamide by Gas Chromatography

2.2.6 *US EPA Method 8033*—Acetonitrile by Gas Chromatography with Nitrogen-Phosphorus Detection

2.2.7 *US EPA Method 8260B*—Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS). The volatile compounds are introduced into the gas chromatograph by direct injection. If the sample can not be dispersed in methanol, which dissolves the volatile organic constituents, a portion of the methanol solution is combined with organic free water in a specifically designed purging chamber. The components are separated via a gas chromatograph and detected with a mass spectrometer, which is used to provide both qualitative and quantitative information. Refer to method write-up for a list of compounds that can be measured by this technique.

07.00 INTRODUCTION TO PATHOGEN TESTING

1. Significance

1.1 Compost is the product of a controlled microbiological breakdown of organic matter. For this reason, composts contain large quantities of bacteria (including actinomycetes) and fungi. As much as 25% of the mass of a finished, mature compost is composed of both the living and non-living cellular material of microbes.

1.2 Composts derived from human or animal wastes may contain pathogenic organisms. If the compost is immature, or if thermophilic conditions are not achieved throughout the composting mass, some pathogenic microbes can survive. Their presence in finished compost that is distributed to or sold for use by the general public must comply with local and state limits and recommendations. For composts that contain biosolids, the US EPA regulation 40 CFR Part 503 stipulates that only products which meet the Class A pathogen limits can be distributed or sold to the general public. In addition, biosolids compost products that only meet the Class B pathogen limit are restricted to use on land at remote sites with appropriate management practices and public access restrictions. Class B biosolids compost testing requirements are presented in Table 07.00-1. Note that reported pathogens test results for Class B compost represent the geometric mean of analytical results from seven representative composite compost samples, whereas the reported pathogens test result for Class A compost may be based upon the analytical result from only one representative composite sample (refer to Section 02.01 Field Sampling of Compost Materials). These limits and practices are designed to protect public health and the environment.

Table 07.00-1 40CFR Part 503 Pathogen density limits for Class B biosolids compost.

Class A Biosolids Pathogen Standard (dw basis).

Pathogen	*Standard Density Limits*
SALMONELLA	< 3 MPN $(4 \cdot g)^{-1}$ TS,
	OR
FECAL COLIFORMS	< 1000 MPN g^{-1} TS, AND
ENTERIC VIRUSES	< 1 PFU $(4 \cdot g)^{-1}$ TS, AND
VIABLE HELMINTH OVA	< 1 $(4 \cdot g)^{-1}$ TS

Class B Biosolids Pathogen Standard (dw basis).

Pathogen	*Standard Density Limits*
Fecal Coliform Density*	< 2,000,000 (MPN or CFU) g^{-1} TS

*The geometric mean of seven samples is reported.

1.3 Local or state regulatory agencies may require that composting operations dealing with compost feedstocks other than biosolids provide assurance that the number of disease causing microorganisms present in finished compost do not exceed specified limits. Check with local and state permitting agencies to determine the limits used in that jurisdiction. Analysis for pathogenic organisms is costly and therefore not required for each batch of product. Time and temperature measurements and records combined with regular but periodic laboratory analyses of samples is sometimes used by regulators as an acceptable alternative. In the interests of time and analytical expense, measurements of indicator organisms are often allowed by regulations as substitutes for direct measurement of pathogenic species.

2. Referenced Documents

2.1 *TMECC:*

Section 02.01 Field Sampling of Compost Materials.

Method 03.09-A Total Solids and Moisture at 70±5°C.

Section 07.01 Coliform Bacteria.

Section 07.02 *Salmonella.*

Section 07.03 *Enterococci.*

Section 07.04 Parasitic Helminths.

Section 07.05 Recovery and Assay of Total Culturable Viruses.

2.2 *Other Sources:*

Control of Pathogens and Vector Attraction in Sewage Sludge. pp. 105-106. *In* EPA Environmental Regulation and Technology (EPA/625/R-92/013).

Sludge Land Application Project: Microbial Procedures Manual. April 1982. King County Metro Document.

SM 9215, Heterotrophic Plate Count. *In* Standard Methods for the Examination of Water and Wastewater. 18th Edition, 1992.

SM 9230 Fecal *Streptococcus* and *Enterococcus* Group. *In* Standard Methods for the Examination of Water and Wastewater. 18th Edition, 1992.

SM 9260D, p. 9-91. Detection of Pathogenic Bacteria. *In* Standard Methods for the Examination of Water and Wastewater. 18th Edition, 1992.

US EPA Code of Federal Regulations. 1993. Standards for the Use or Disposal of Sewage Sludge. Title 40, Volume 3, Parts 425 to 699, Federal Register February 19, 1993 (58 FR 9248), U.S. Government Printing Office. Washington, D.C. [40CFR503.3] .

US EPA Method 9131 Total Coliform—Multiple Tube Fermentation Technique. *In* Test Methods for Evaluating Solid Waste. Volume 1B. Laboratory Manual Physical/Chemical Methods. (SW-846) 1992.

US EPA Process to Further Reduce Pathogens (PFRP). US EPA 40 Code of Federal Regulation Part 503.

3. Terminology

3.1 *autoclave, n*—A strong, pressurized, steam-heated vessel used for sterilization of laboratory media, glassware and utensils.

3.2 *autotroph, n*—An organism capable of synthesizing its cellular components from inorganic substances, using light or chemical energy. Green plants, algae, and certain bacteria are autotrophs.

3.3 *bacteria, n pl*—Any of the unicellular microorganisms of the kingdom Procaryotae which vary in terms of morphology, oxygen and nutritional requirements, and motility, and may be free-living, saprophytic, or pathogenic.

3.4 *coliform, adj*—Of or relating to the bacilli that commonly inhabit the intestines of human beings and other vertebrates, especially the colon bacillus, *Escherichia coli*.

3.5 *fecal, adj*—Of, relating to, or composed of waste matter eliminated from the bowels.

3.6 *fungi, n pl*—Any of numerous eukaryotic organisms of the kingdom Fungi, which lack chlorophyll and vascular tissue and range in form from a single cell to a mass of branched filamentous hyphae (mycelia) that produce specialized fruiting bodies. The kingdom includes the yeasts, , smuts, rusts, and mushrooms.

3.7 *heterotroph, n*—An organism that is dependent on complex organic substances for synthesizing cellular components.

3.8 *helminth, n*—A worm, especially a parasitic roundworm (nematode) or tapeworm.

3.9 *pathogen, n*—An agent that causes disease, such as a bacterium, fungus, helminth, protozoan, or virus.

3.10 *protozoa, n*—Any of a large group of single-celled, usually microscopic, eukaryotic organisms, such as amoebas, ciliates, flagellates, and sporozoans.

3.11 *saprophyte, n*—An organism, that does not cause disease or require another living organisms as its host for growth.

3.12 *virus, n*—Any of various simple submicroscopic parasites of plants, animals, and bacteria that often cause disease and that consist essentially of a core of RNA or DNA surrounded by a protein coat. They are unable to replicate without a host cell and are often referred to as obligate intracellular parasites.

4. Sample Handling

4.1 Samples at as-received moisture content are used for pathogen tests. Sample moisture content is determined on a parallel sample aliquot and is used to correct reported values from as-received moisture basis to dry weight basis, i.e., total solids basis (Method 03.09-A). If delays in isolation are anticipated, store compost samples in sealed containers at approximately 4°C. Refer to Table 07.00-2 for more specific information on sample containers, conditions and holding times. The most important considerations with regard to sample containers is their cleanliness and sterility and ability to withstand the rigors of express shipping. Plastic bags should be at least 4 mil thickness and double bagged for shipment. Jars should have a wide-mouth opening. Because of the high ratio of coliform bacteria to pathogens, large compost samples (1,000 cm^3) are required.

4.2 It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use. Aseptic techniques and sterile materials and apparatus should be used throughout all methods in this section.

Table 07.00-2 *Pathogens:* Sample containers and conditions for compost and feedstock testing.

Test Parameter of Interest	*Container*	*Conditions*	*Maximum Holding Time in Lab*
Enteric Virus	G	-70°C	> 8 hours
Enteric Virus	SP, G	4°C	8 hours
Coliforms and other bacteria	SP, G	4°C	48 hours
Helminth Ova	SP, G	4°C	1 month

NOTE 1—SP=Sterilized Polypropylene; G= Sterilized Glass

5. Washing, Sterilization and Disposal

5.1 Cleanse all glassware thoroughly with a suitable detergent and hot water, rinse with hot water to remove all traces of residual washing compound, and finally rinse with laboratory-pure water. If mechanical glassware washers are used, equip them with influent pumping of stainless steel or other nontoxic material. Do not use copper piping to distribute water. Use stainless steel or other nontoxic material for the rinse water system.

5.2 Sterilize glassware that contains no liquid media or water, except when in metal containers, for not less than 60 min at a temperature of 170°C, unless it is known from recording thermometers that oven temperatures are uniform, under which exceptional conditions use 160°C. Heat glassware in metal containers to 170°C for not less than 2 h.

5.3 Sterilize all sample bottles by autoclaving at 121°C for 15 min.

5.3.1 For all containers loosen caps before autoclaving to prevent distortion and to allow steam flow.

5.4 *Disposal*—Autoclave all contaminated media for 30 min before discarding. All caps on containers and tubes must be secure but open enough for steam to escape. After use all units must be autoclaved at 121°C for 30 min before disposal.

6. Keywords

6.1 *Ascaris* ova; autoclave; autotroph; bacteria; coliform; *Enterococcus*; fecal; fungi; heterotroph; pathogen; protozoa; *Salmonella*; saprophyte; virus

Test Method: Coliform Bacteria. Three Methods							**Units:** MPN g^{-1}, dw basis	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							07.01-A	
							07.01-B	
							07.01-C	

07.01 COLIFORM BACTERIA

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org/addenda.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section covers detection and quantification techniques for coliform bacteria.

1.1.1 *Method 07.01-A Total Coliforms.*

1.1.2 *Method 07.01-B Fecal Coliforms*.

1.1.3 *Method 07.01-C Escherichia coli*.

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 03.09-A Total Solids and Moisture at 70±5°C

2.2 *Other Sources:*

Eaton, A.D., L.S. Clesceri, and A.E. Greengerg, Standard Methods for the Examination of Water and Wastewater. 19th ed., ed. A.E. Greenberg. 1995, Washington, D.C.: American Public Health Association. 539.

Murray, P.R., Medical Microbiology. 3rd ed. 1998, St. Louis, MO. Mosby. x, 719.

Joklik, W.K., *et al*., Zinsser Microbiology. 20th ed. 1992, Norwalk: Appleton & Lange. 1294

3. Terminology

3.1 *coliform, n*—a lactose-fermenting member of the family Enterobacteriaceae. commonly associated with the intestinal tract of animals, including humans, fish, birds and insects. However, many are also known and reported to be free-living in the environment and associated with plants and soil. While most coliforms are not medically significant, all are opportunistic pathogens and able to cause disease in the very young and old, and immunologically compromised individuals.

3.2 *ten-fold dilution series*, *n*—a one mL aliquot of a sample is diluted to ten mL and one mL of each dilution is inoculated into a separate tube of broth for incubation. Refer to Fig 07.01-1.

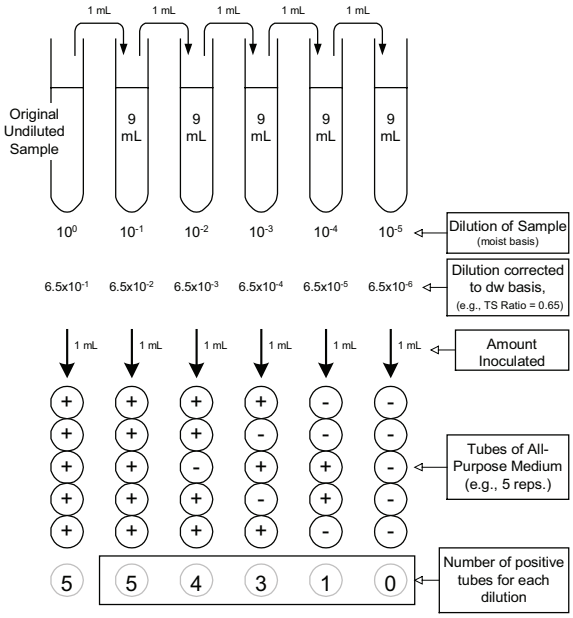

Fig 07.01-1. Conceptual diagram of a five-tube ten-fold dilution series with five incubation replicates per dilution. Note that sample dilutions are converted to dry weight basis before calculating the MPN. The illustration includes a hypothetical test result (box at bottom of figure) used when calculating MPN.

3.3 *fecal coliforms, n*—indicator organisms for fecal pathogens; a subset of coliforms (lactose-fermenting enterics) that are considered to be associated almost exclusively with the intestinal tracts of animals and insects (few strains are associated with plant material).

3.4 *Escherichia coli, n*—the classical example of a fecal coliform; found in feces from all animals, hence its presence in compost is evidence of fecal contamination. Most strains of *E. coli* are opportunistic pathogens and are unable to cause disease in healthy humans. However, some strains are pathogenic. Good compost manufacturing procedures are able to reduce the numbers of *E. coli* in the final product to a level that will protect public health and the environment. Specific time-temperature conditions for the various methods of composting must be met in order to achieve pathogen reduction and satisfy any standard limits that jurisdictions may impose.

3.5 *indicator organism, n*—microbes that are generally not pathogenic, but co-exist in habitats with pathogens. Detection and quantification of an indicator organism in a sample is presumptive evidence that pathogens may also be present in the habitat from which the sample was obtained. Detection and quantification of indicator organisms is often much simpler and less costly than detecting/quantifying specific pathogens.

4. Summary of Test Methods

4.1 *Method 07.01-A Total Coliforms*—The method described herein will determine the presence and quantity of total coliforms in a compost sample. Combined application of two traditional culture methods, spread plating and a Most Probable Number method (MPN), facilitates the quantification of coliforms over a very broad range[1], from less than 3.6 MPN g^{-1} to 10^6 cfu g^{-1} (dw basis), while surpassing several inherent limitations associated with each method.

4.2 *Method 07.01-B Fecal Coliforms*—The method described herein will determine the presence and quantity of fecal coliforms in compost. Combined application of two traditional culture methods, spread plating and MPN, facilitates the quantification of fecal coliforms over a very broad range[1], from less than 3.6 MPN g^{-1} to 10^6 cfu g^{-1} (dw basis), while surpassing several inherent limitations associated with each method.

4.3 *Method 07.01-C Escherichia coli*—This method described herein will determine the presence and quantity of *E. coli* in compost. Combined application of two traditional culture methods, spread plating and MPN, facilitates the quantification of fecal coliforms over a very broad range[1], from less than 3.6 MPN g^{-1} to 10^6 cfu g^{-1} (dw basis), while surpassing several inherent limitations associated with each method.

5. Significance and Use

5.1 *Method 07.01-A Total Coliforms*—The detection of coliforms indicates the possible presence of enteric pathogens. A finding of total coliforms indicates that the compost does not contain growth inhibitors for enteric bacteria. Many coliforms are not exclusively associated with fecal material; their presence should not be used or interpreted as an indication of the presence of pathogens. Lauryl Tryptone Broth (LT) is used as a non-selective, resuscitative medium for quantification of total coliforms in the coliform MPN (detects <3.6 – 11,000 MPN g^{-1}). MacConkey's agar is used as a selective and differential medium for the quantification of total coliforms when incubated at 35°C for 18 h – 24 h. Spread plate counts may provide quantification information between 10^3 – 10^6 cfu g^{-1}.

5.2 *Method 07.01-B Fecal Coliforms*—Fecal coliforms are indicators of fecal contamination. A finding of fecal coliforms indicates that pathogens may be present in the sample. Fecal coliforms have the distinction of growing and surviving at higher temperatures and in the presence of bile salts than other coliforms. The EC-MUG medium, which contains bile salts, is used for selective growth and enumeration of fecal coliforms by the MPN technique (07.01-B). It is incubated at 44.5°C ± 0.2°C to enhance the selectivity. Duplicate MacConkey's agar plates also may be used to estimate cfu of fecal coliforms when incubated at 44.5°C ± 0.2°C. Fecal coliforms are significantly reduced during the thermophilic phase of the composting process. Fecal coliforms are quantified in the finished compost to indicate that the composting procedures have effectively destroyed large numbers of enteric pathogens. It is important to recognize that the presence *per se* of fecal coliforms in compost does not mean that the compost is unfit for use. For example, amounts of fecal coliforms up to 2,000,000 MPN per gram total solids in biosolids compost are considered by the US EPA (40CFR Part 503) to be acceptable for land application at remote agricultural sites where public access is restricted and specific management practices are observed. For biosolids compost to be distributed to the general public without restriction, fecal coliforms may not exceed 1,000 MPN per gram total solids in the final product according to provisions of 40CFR Part 503.

5.3 *Method 07.01-C Escherichia coli—E. coli* is the predominant fecal coliform found in human and animal fecal matter. Its presence indicates the potential presence of enteric pathogens. A finding of *E. coli* in compost must be interpreted in the context of the concentration. The presence of trace amounts of *E.*

[1] The power of the MPN method for food, soil, compost, and water analyses increases with the number of analyses performed per sample. The 3-tube method described here is easily be extended to a 5 or 10 tube method with a corresponding increase in the power (accuracy) of the estimate.

coli can sometimes be found in finished compost that has been properly processed. However, trace amounts in a few samples simply may indicate that contamination (from birds or other wildlife) may have occurred after the compost completed proper temperature cycling. The *E. coli* MPN (17.01°C; 19.0) system is performed using same medium provided by the fecal coliform MPN (17.01°C; 14.0). Simply observing the positive fecal coliform MPN tubes under a long-wave ultraviolet (~365 nm) light source will determine if *E. coli* is present. *E. coli* produces a fluorogenic compound by cleaving the substrate 4-methyllumbelliferyl-beta-D-glucuronide (MUG).

6. Interference and Limitations

6.1 *Method 07.01-A Total Coliforms*—Most Probable Number (MPN) methods have several limitations including the requirement of time, effort and equipment required to handle large quantities of materials per sample. Direct plating onto very selective and differential media, (e.g., MacConkey's agar), has been reported to be inefficient in growing organisms that have been injured or are described as being viable, but not culturable. The strategy proposed here that involves simultaneous use of spread plates and a limited MPN was designed to avoid the massive supply needs of a broad dilution range MPN while eliminating the low sensitivity and cultivability problems with spread plates.

6.2 *Method 07.01-B Fecal Coliforms*—The MPN methods have several limitations including the requirement of time, effort and supplies required to handle large quantities of materials per sample. Direct plating onto very selective and differential media, (e.g., MacConkey's agar), has been reported as inadequate for resuscitation and growth of cells that have been injured and remain viable but not culturable, especially with growth at 44.5°C. The strategy proposed here involves the simultaneous use of spread plates and a limited MPN. This dual approach avoids the need for large input of supplies for the MPN while eliminating the low sensitivity and cultivability problems with spread plates. The fecal coliform MPN uses Lauryl-Tryptone broth (LT, from Method 07.01-A, step 10.0) as a non-selective enrichment prior to inoculating the sample in EC-MUG, a selective and differential medium for fecal coliforms and *E. coli.* This technique allows the resuscitation of any injured organisms and eliminates the possibility of including auto-fluorescent materials into the EC-MUG medium, which would interfere with the interpretations of the test.

6.3 *Method 07.01-C Escherichia coli*—This test, which is a supplemental method to the fecal coliform method, is based on the ability of *E. coli* to produce the enzyme β-glucuronidase (GUD). 94% of *E. coli* have been reported to produce GUD. GUD cleaves the substrate 4-methylumbelliferone-β-D-Glucuronide (MUG), producing 4-methylumbelliferone (MU) which is fluorescent under long wave ultraviolet light (465 nm). A limitation of this method includes the possibility 'counting' false positive organisms based solely on the ability to fluorescence. However, most fluorescent organisms (other than *E. coli*) will not grow under the growth conditions and growth medium described in the methods, i.e. EC-MUG medium at 44.5°C ± 0.2°C. It must be noted that this method will not detect *E. coli* O157:H7, a serotype responsible for significant foodborne illnesses. *E. coli* O157:H7 has been reported to be sensitive to elevated temperatures, 44.5°C, and does not produce the GUD enzyme required to make the medium fluoresce.

7. Sample Handling

7.1 Samples at as-received moisture content are used for these tests. Moisture analysis of a parallel sample aliquot must be conducted so that reported data can be corrected to a dry weight basis, (refer to Method 03.09 Total Solids and Moisture). If delays in analysis are anticipated, store compost samples in sealed containers at approximately 4°C. Large compost samples must be homogenized and mixed thoroughly before the subsamples for microbial analysis are collected. Thorough mixing helps overcome heterogeneous distribution of microbes.

Test Method: Coliform Bacteria. Total Coliforms							**Units:** MPN g^{-1}	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							07.01-A	

07.01-A TOTAL COLIFORMS

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

SUBMITTED BY—Soil Microbial Systems Laboratory; USDA-ARS-BARC; Beltsville, MD 20705

8. Apparatus for Method A

8.1 *Most Probable Number Technique:*

8.1.1 *culture tubes*—16-mm × 150-mm, screw-top tubes (e.g., Fisherbrand).

8.1.2 *dilution tubes*—16-mm × 150-mm, screw-top tubes filled with 9ml Buffered Peptone Water

8.1.3 *incubator*—set at 36°C ± 1°C.

8.1.4 *inverted gas tubes*—6-mm × 50-mm, (e.g., Fisherbrand).

8.1.5 *strainer bag*—sterile stomacher bag, (e.g., Stomacher Model 400C, Seward Medical).

8.2 *Spread Plating:*

8.2.1 *spiral-plating machine*—optional alternative to conventional spread plating method, (e.g., Spiral Biotech).

8.2.2 *agar plates*—MacConkey's Agar, (e.g., Becton Dickinson)

8.2.3 *hockey sticks*—bent glass rods used to spread organisms onto the agar surface

8.2.4 *bunsen burner*

9. Reagents and Materials for Method A

9.1 *Most Probable Number Technique:*

9.1.1 buffered peptone water—BPW, (e.g., Becton Dickinson).

9.1.2 Lauryl-Tryptose broth—LT, (e.g., Becton Dickinson).

9.2 *Spread Plating:*

9.2.1 *ethanol*—70% for sterilization.

10. Procedure for Method A

10.1 *Most Probable Number Technique:*

10.1.1 *Prepare 10^{-1} Homogenate*—Place 20 g of compost into sterile stomacher bag. Bring weight up to 200 g with the addition of buffered peptone water (BPW) for a 1:10 dilution (10^{-1}).

10.1.1.1 Homogenize for 2 min at 260 rpm.

10.1.1.2 Prepare three additional dilutions by making three 1:10 serial dilutions in sterile BPW. This is done by adding 1 mL sample homogenate (10^{-1}) to 9 mL BPW, vortexing for 5-10 sec, and continuing this dilution process two more times.

10.1.1.3 Prepare at least nine screw-top culture tubes (e.g., three dilutions in triplicate, etc.), each containing 9 mL sterile Lauryl-Tryptose broth (LT) each containing an inverted gas tube.

10.1.1.3.1 Aseptically transfer 1 mL of the 1:10 (10^{-1}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile LT.

10.1.1.3.2 Aseptically transfer 1 mL of the 1:100(10^{-2}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile LT.

10.1.1.3.3 Aseptically transfer 1 mL of the 1:1000 (10^{-3}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile LT.

10.1.2 Incubate tubes for 24 h to 48 h in a 37° ± 2 °C incubator.

10.1.3 Observe the inverted gas tubes for the presence of small air bubbles. Gas formation indicates a 'positive' result for lactose fermentation, and is therefore a positive result for a coliform. Record the number of tubes in each dilution set that are positive for gas formation. Convert dilutions to a dry weight basis by multiplying tube concentration by the total solids ratio as determined on a parallel aliquot (Method 03.09-A). This number will be used to calculate the MPN g^{-1} for total coliforms (Equation Step 11.1).

10.2 *Spread Plating Technique:*

10.2.1 Prepare one MacConkey's agar plate if using an automated spiral-plating machine, or six plates if using standard spread plating techniques. Air-dry the surface of the plates by maintaining them covered at room temperature for one day, or place into a laminar flow hood for 10 minutes with the lids ajar.

10.2.2 If using an automated spiral-plating machine, plate 50µL from the 10^{-2} dilution used during the MPN protocol above. Incubate for 18 to 24 h at 37 ± 1°C.

10.2.3 If using standard spread plate methods, place 100 µL from the 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions onto the surfaces of different MacConkey plates. Spread the liquid evenly onto the surface of each agar plate using a sterilized glass 'hockey-stick.' The sample-spreading procedure is facilitated by the use of a rotating Petri-dish holder. The hockey sticks are sterilized easily by dipping in 70% ethanol and briefly passing through a flame to eliminate trace residue of the alcohol. Incubate all plates at 37°C ± 1°C for 18 h - 24 h.

10.2.4 Observe the agar surface for colonies that are bright pink or red. These colonies are considered to be coliforms (gram negative, lactose-fermenting members of the family Enterobacteriaceae).

11. Calculations for Method A

11.1 *Most Probable Number Technique*—Record the number of positive tubes in each dilution set. Select the highest dilution that gives positive results in all tubes (even if a lower dilution gives negative results), plus the next two higher dilutions. For dilutions prepared with 'as received' samples, i.e., wet weight basis, convert the test aliquot size from wet weight basis to dry weight basis by multiplying each dilution times the total solids ratio (refer to Method 03.09-A), and compute the MPN g^{-1} dw using the *MPN Calculator,* available on-line at http://tmecc.org/mpn/.

11.2 *Colony Forming Units Technique (CFU)*—Quantify the total coliforms as colony-forming units (cfu g^{-1} dw) using the protocols included by the manufacturer of the spiral-plating equipment used. If using standard spread plating techniques, count the pink colonies and perform the calculation using the following formula:

$$CFU = (C \div V) \times D \times TS \qquad \textit{Equation 11.2}$$

where:

CFU = colony forming units per gram of sample; number of cells in original sample, cfu g^{-1} dw,

C = number of colonies of the target organism, (e.g., coliforms = pink or red colonies),

V = volume plated, mL, i.e., 100µL = 0.1 mL; 50 µL = 0.05 mL, etc.,

D = dilution factor, mL g^{-1}; (e.g., 1 ÷ dilution, i.e., 1 ÷ 10^{-2} = 10; 1 ÷ 10^{-4} = 1,000, etc.), and

TS = total solids ratio = mass of oven dried aliquot ÷ mass of aliquot at as-received moisture, (refer to Method 03.09-A).

Test Method: Coliform Bacteria. Fecal Coliforms							**Units:** MPN g^{-1}	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							07.01-B	

07.01-B FECAL COLIFORMS

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

SUBMITTED BY—Soil Microbial Systems Laboratory; USDA-ARS-BARC; Beltsville, MD 20705

12. Apparatus for Method B

12.1 *Most Probable Number Technique:*

12.1.1 *culture tubes*—16-mm × 150-mm, screw-top tubes (e.g., Fisherbrand).

12.1.2 *dilution tubes.*

12.1.3 *incubator*—set at 35°C to 37ºC.

12.1.4 *inverted gas tubes*—6-mm × 50-mm, (e.g., Fisherbrand).

12.1.5 *strainer bag*—sterile stomacher bag, (e.g., Stomacher Model 400C, Seward Medical).

12.2 *Spread Plating:*

12.2.1 *spiral-plating machine*—optional, (e.g., Spiral Biotech).

12.2.2 *agar plate*—MacConkey's, (e.g., Becton Dickinson).

12.3 *Flame*—Alcohol or gas.

13. Reagents and Materials for Method B

13.1 *Most Probable Number Technique:*

13.1.1 *buffered peptone water*—BPW, (e.g., Becton Dickinson).

13.1.2 EC-MUG—*E. Coli* Medium plus 4-methylumbelliferone-β-D-Glucuronide (e.g., Becton Dickinson).

13.2 *Spread Plating:*

13.2.1 *ethanol*—70% for sterilization.

13.2.2 *glass hockey sticks.*

14. Procedure for Method B

14.1 *Most Probable Number Technique:*

14.1.1 Prepare at least nine screw-top culture tubes, (e.g., three dilutions in triplicate, etc.), each containing 9 mL sterile *E. coli* Medium plus MUG (EC-MUG).

14.1.2 For each positive LT tube (from step 10.12), aseptically transfer 20 µL – 40 µL into a culture tube containing 9 mL EC-MUG.

14.1.3 Incubate all EC-MUG tubes at 44.5°C ± 0.2°C for 18 h to 24 h.

14.1.4 Observe the EC-MUG gas tubes for presence of air bubbles. Gas formation indicates a 'positive' result from lactose fermentation. All EC-MUG tubes that contain gas are considered POSITIVE for growth of fecal coliforms. Convert dilutions to a dry weight basis by multiplying tube concentration by the total solids ratio as determined on a parallel aliquot (Method 03.09-A). Record the number of positive tubes in each dilution, as this number will be used to calculate the MPN g^{-1} dw for fecal coliforms (step 15.1).

14.2 *Spread Plating Technique:*

14.2.1 Prepare one MacConkey's agar plate if using an automated spiral-plating machine, or six plates if using standard spread plating techniques. Air dry the surface of the plates by maintaining them at ambient laboratory temperature for one day, or place in a laminar flow hood for 10 min with the lids removed.

14.2.2 If using an automated spiral-plating machine plate 50 µL from the 10^{-2} sample dilution. Incubate for 18 h to 24 h at 44.5°C ± 0.2°C.

14.2.3 If using standard spread plate methods, place 100 µL from the 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions onto the surfaces of a different MacConkey plates. Spread the liquid evenly onto the surface of each agar plate using a glass 'hockey-stick' that has been dipped in 70% ethanol and briefly passed under a flame. The sample-spreading procedure is facilitated by the use of a rotating Petri-dish holder. Incubate all plates at 44.5°C ± 0.2°C.

15. Calculations for Method B

15.1 *Most Probable Number Technique*—Record the number of positive tubes in each dilution set. Select the highest dilution that gives positive results in all tubes, (even if a lower dilution gives negative results), plus the next two higher dilutions. For dilutions prepared with 'as received' samples, i.e., wet weight basis, convert the test aliquot size from wet weight basis to dry weight basis by multiplying each dilution times the total solids ratio (refer to Method 03.09-A), and compute the MPN using the *MPN Calculator,* available on-line at http://tmecc.org/mpn/. Report as

MPN g^{-1} dw basis to comply with reporting requirements for biosolids composts as indicated under US EPA 40CFR 503.

15.2 *Colony Forming Units Technique (CFU)*— Quantify the fecal coliforms (cfu g^{-1} dw) using the protocols provided by the manufacturer of the spiral-plating equipment used. If using standard spread plating techniques, count the pink colonies and perform the calculation using the following formula:

$$CFU = (C \div V) \times D \times TS \qquad \textit{Equation 15.2}$$

where:

CFU = colony forming units per gram of sample; number of cells in original sample, cfu g^{-1} dw,

C = number of colonies of the target organism, (e.g., coliforms = pink or red colonies),

V = volume plated, mL, i.e., 100µL = 0.1 mL; 50 µL = 0.05 mL, etc.,

D = dilution factor, mL g^{-1}; (e.g., 1 ÷ dilution, i.e., 1 ÷ 10^{-2} = 10; 1 ÷ 10^{-4} = 1,000, etc.), and

TS = total solids ratio = mass of oven dried aliquot ÷ mass of aliquot at as-received moisture, (refer to Method 03.09-A).

Test Method: Coliform Bacteria. *Escherichia coli*							**Units:** MPN g^{-1}	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
							07.01-C	

07.01-C *ESCHERICHIA COLI*

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

SUBMITTED BY—Soil Microbial Systems Laboratory; USDA-ARS-BARC; Beltsville, MD 20705

16. Apparatus for Method C

16.1 *culture tubes*—16-mm × 150-mm, screw-top tubes (e.g., Fisherbrand).

16.2 *dilution tubes.*

16.3 *incubator*—set at 35°C to 37ºC.

16.4 *inverted gas tubes*—6-mm × 50-mm, (e.g., Fisherbrand).

16.5 *strainer bag*—sterile stomacher bag, (e.g., Stomacher Model 400C, Seward Medical).

16.6 *flame*—Alcohol or gas.

17. Reagents and Materials for Method C

17.1 EC-MUG—*E. coli* Medium plus MUG, (e.g., Becton Dickinson).

17.2 MacConkey's Agar (MAC).

17.3 Eosin-Methylene Blue Agar (EMB, Becton Dickinson) plates.

17.4 Indole reagent (e.g., Becton Dickinson).

17.5 Triple Sugar Iron Agar (TSI, e.g., Becton Dickinson) slant.

17.6 Motility Indole Lysine Agar (MIL) deep.

17.7 Glass hockey sticks.

18. Procedure for Method C

18.1 *Most Probable Number Technique:*

18.1.1 Prepare at least nine screw-top culture tubes, (e.g., three dilutions in triplicate, etc.), each containing 9 mL sterile *E. coli* Medium plus MUG (EC-MUG).

18.1.2 For each positive LT tube (from step 10.12), aseptically transfer 20µL into a culture tube containing 9mL EC-MUG.

18.1.3 Incubate all EC-MUG tubes at 44.5°C ± 0.2°C. for 18 h - 24 h.

18.2 Observe the EC-MUG tubes (from Method 07.01-B, step 14.1.4, fecal coliform) under long wave ultraviolet light (~ 365 nm). Any tube that fluoresces AND that contains gas in the inverted gas tube is considered POSITIVE for *Escherichia coli.* Note the number of tubes that fluoresce AND contain gas in each dilution. Convert dilutions to a dry weight basis by multiplying tube concentration by the total solids ratio as determined on a parallel aliquot (Method 03.09-A). This number will be used to calculate the MPN g^{-1} for presumptive *Escherichia coli* (Step 20.1).

18.3 Each presumptive positive tube for *Escherichia coli* should be confirmed.

19. Biochemical Confirmation of *Escherichia coli*

19.1 Prepare three MacConkey's Agar (MAC) and three Eosin-Methylene Blue Agar (EMB) plates. Use one MAC and one EMB plate per each dilution set. Divide each plate into three sections, labeled A, B and C so that each tube within each dilution set has a corresponding section on both EMB and MAC plates.

19.2 Using a sterile loop, remove one loopful of culture from each positive EC-MUG tube and, using the same loop, streak for isolation on both identically labeled MAC and EMB sections.

19.3 Incubate plates at 36°C ± 1°Cfor 18 h - 24 h.

19.4 *Observe growth—Escherichia coli* produces a deep pink coloration on MAC plates, and the medium surrounding this culture should have a 'fuzzy' pink appearance due to the precipitation of bile salts and low pH (due to lactose fermentation). Growth on EMB should be metallic green within 18 h – 24 h, but can also appear dark purple.

19.5 Prepare one Triple Sugar Iron Agar (TSI) slant one Motility Indole Lysine Agar (MIL) deep for each isolate to be tested.

19.5.1 Pick three colonies from the MAC plate that are pink, have precipitated bile salts AND that have a corresponding sector of EMB that has a metallic green sheen (or appears dark purple). Using the same needle for each medium, streak the surface and then stab the bottom of the TSI tube, then stab the MIL tube twice.

19.5.2 Incubate the TSI and MIL tubes for 18 h - 24 h at 36°C ± 1°C. Place two drops of Indole reagent onto the surface of each MIL tubes. DO NOT SHAKE THE TUBE.

19.6 *Escherichia coli* should exhibit the following biochemical characteristics:

19.6.1 *TSI*—Acid Slant (A/Yellow), Acid Butt (A/Yellow), Gas production (bubbles) throughout the medium.

19.6.2 *MIL*—Basic Slant (K/Purple), Basic Butt (K/Purple), Motility (medium is cloudy), and Indole production (Red band at the top of the tube, after the addition of Kovac's Reagent).

20. Calculations for Method C

20.1 *Most Probable Number Technique*—Record the number of positive tubes in each dilution set. Select the highest dilution that gives positive results in all tubes (even if a lower dilution gives negative results), plus the next two higher dilutions. For dilutions prepared with 'as received' samples, i.e., wet weight basis, convert the test aliquot size from wet weight basis to dry weight basis by multiplying each dilution times the total solids ratio (refer to Method 03.09-A), and compute the MPN g^{-1} dw using the *MPN Calculator,* available on-line at http://tmecc.org/mpn/.

07.01 METHODS SUMMARY

21. Report

21.1 *Method 07.01-A Total Coliforms*—Depending on the concentration of coliforms in the sample, results will be reported in either cfu g^{-1} dw (from spread plating data) or MPN g^{-1} dw (from Most Probable Number multiple tube tests); or both. Results should be reported on a dry weight (dw) basis for all quantification tests.

21.2 *Method 07.01-B Fecal Coliforms*—Depending on the concentration of fecal coliforms in the sample, results will be reported in either cfu g^{-1} dw (from spread plate data of colony counts) or MPN g^{-1} dw (from Most Probable Number multiple tube tests); or both. Results should be reported on a dry weight (dw) basis for all quantification tests. Report as MPN g^{-1} dw basis to comply with reporting requirements for biosolids composts as indicated under US EPA 40 CFR 503.

21.3 *Method 07.01-C Escherichia coli—E. coli* presence will be reported as the MPN g^{-1} dw basis for all quantification tests. Report confirmation tests for *E. coli.* The MPN result may need to be adjusted according to the number of tubes that were confirmed biochemically.

22. Precision and Bias

22.1 The precision and bias of the tests listed below have not been determined. Data are being sought for use in developing a precision and bias statement.

22.1.1 *Method 07.01-A Total Coliforms*

22.1.2 *Method 07.01-B Fecal Coliforms*

22.1.3 *Method 07.01-C Escherichia coli*

23. Keywords

23.1 coliform; fecal coliform; *Escherichia coli*, most probable number; MPN; spread plating; compost, quantification.

Test Method: *Salmonella.* Three Methods							**Units:** MPN $(4 \cdot g)^{-1}$, dw basis	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	07.02-A	07.02-A		07.02-A			07.02-A	
	07.02-B	07.02-B		07.02-B			07.02-B	
	07.02-C	07.02-C		07.02-C			07.02-C	

07.02 SALMONELLA

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This test covers the detection and quantification techniques for *Salmonella spp*. in compost samples

1.1.1 *Method 07.02-A 1-2 Detection Test and Quantification Procedure for Salmonella in Compost.*

1.1.2 *Method 07.02-B Enrichment and Quantification of Salmonellae in Compost.*

1.1.3 *Method 07.02-C Confirmation Protocols for Presumptive Salmonella Isolates.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 03.09-A Total Solids and Moisture at 70±5°C

2.2 *Other Sources:*

Method 9260D, Detection of Pathogenic Bacteria. p. 9-91. *In* Standard Methods for the Examination of Water and Wastewater. 18th Edition, 1992.

3. Terminology

3.1 *Salmonella, n*—Any of various rod-shaped bacteria of the genus *Salmonella*, all of which are pathogenic to animals and humans, causing food-borne illnesses, typhoid, and paratyphoid fever.

4. Summary of Test Methods

4.1 *Method 07.02-A 1-2 Detection Test and Quantification Procedure for Salmonella in Compost*— The test is based on the observation of well-defined bands of salmonellae cells (ImmunoBand) that have become immobilized in motility medium by polyvalent H (flagellar) antibodies. The quantification procedure is based on a Most-Probable Number MPN format.

4.2 *Method 07.02-B Enrichment and Quantification of Salmonellae in Compost*—A two-step method is used to ensure the detection of viable *Salmonella spp.* in compost.

4.2.1 *Enrichment procedure*—Because of the time, effort and expense of this quantification method, a preliminary detection screening analysis is first conducted to rule out 'negative' samples prior to processing the samples through the quantification system. The first step is to perform an enrichment procedure to detect a single, viable cell of *Salmonella* in a 20-g sample (Detection limit: 0.04 cfu g^{-1}). If any viable cells are recovered and confirmed as salmonellae, then an additional 20 g is used to quantify how many cells per gram of salmonellae are in the sample.

4.2.2 *Quantification procedure*—This strategy uses a three-tube MPN for quantifying low numbers (3.6 – 11,000 cfu g^{-1}), as well as a spread plating technique that can quantify larger numbers (2×10^3 cfu g^{-1} to 2×10^8 cfu g^{-1}) of *Salmonella* spp. should they be present in the compost sample. The MPN protocol uses a multistep system for resuscitating, selectively enriching, and simultaneously quantifying *Salmonella spp*. in compost. It is a five-day procedure. Spread plating requires only two days (one day to perform and a second day to confirm isolates). The plating system is not as robust for compost, manure, and biosolids samples, because it subjects bacterial cells that may be injured, but viable, to growth stressors that are included in the agar media to improve selectivity for salmonellae where many different groups of microorganisms potentially co-exist the sample. The MPN system can be adapted to enumerate larger

numbers of organisms by adding more dilutions and more tubes. Because of the labor and time requirement for processing many samples, the MPN protocol commonly is limited to a 3-tube four dilution level system to detect and enumerate lower numbers of organisms whereas the spread plating system is preferred for detection and enumeration of larger numbers of organisms.

5. Significance and Use

5.1 All *Salmonellae* are considered true pathogens, i.e., any viable cells of *Salmonella* spp. found in the finished compost are potentially pathogenic to animals or humans. Because *Salmonella* spp. is among the leading causes of foodborne illness in the United States, any potential sources of food-supply contamination should be examined and eliminated. This is the overall rationale behind the standards (< 3 salmonellae per 4 g of total solids (dw basis), see Table 07.00-1) that the US EPA has established for public distribution of composted biosolids. The relevant 40CFR part 503 standards are designed to protect the public health and environment relative to risk of illness from contact with fecal pathogens.

5.2 Salmonellae are very susceptible to heat and other environmental stressors such as low moisture and low water activity (A_w). For these reasons, the presence of viable salmonellae in finished compost indicates that the compost has either not been properly heat pasteurized, or that the 'finished' compost has been re-inoculated from some outside source, (e.g. rodent or other animal or bird droppings or contaminated equipment).

5.3 It is important to note that finding salmonellae in compost does not imply that the compost is hazardous. The amounts present and the manner in which compost will be distributed and used are important determinants in the outcome. The presence of this pathogen in soil or composts must be considered carefully when deciding to use the product in ways that may lead to its contact with water or foods since these are two pathways that can lead to disease.

5.4 Several conditions must be met in order for disease to occur:

5.4.1 the pathogen must reach a susceptible host;

5.4.2 the pathogen must be ingested in sufficient quantity to cause disease; and

5.4.3 the person must be susceptible to the amount ingested. For salmonellae, thousands of cells are needed in order for the organism to cause disease in humans.

5.5 In the case of post-consumer cafeteria residues, pathogens present in low numbers initially may increase their populations on the residuals between the time of collection and the start of composting. As with other feedstocks potentially contaminated with pathogens, every effort must be made to destroy them or reduce their populations to virtually undetectable levels so the final compost can be distributed and used safely by the general public (this would include use in bulk landscaping situations where the public may come into contact with the product).

5.6 Samples that are 'positive' for salmonellae contamination should be re-composted, re-tested, and determined to meet the US EPA standard before release or sale to the general public or before use in production of fresh market vegetables and fruits that might contact the compost during growth.

6. Sample Handling

6.1 Samples at as-received moisture content are used for this test. Moisture analysis of a parallel sample aliquot must be conducted so that reported data can be corrected to a dry weight basis, (refer to Method 03.09 Total Solids and Moisture). If delays in isolation are anticipated, store compost samples in sealed containers at approximately 4°C. Because of the high ratio of coliform bacteria to pathogens, large compost samples (1 L or 1,000 cm^3) are required.

Test Method: *Salmonella.* 1-2 Detection Test and Quantification Procedure for *Salmonella* in Compost							**Units:** MPN $(4 \cdot g)^{-1}$, dw basis	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	07.02-A	07.02-A		07.02-A			07.02-A	

07.02-A 1-2 DETECTION TEST AND QUANTIFICATION PROCEDURE FOR *SALMONELLA* IN COMPOST

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

SUBMITTED BY—Avocet Environmental Testing. 1500 North State Street, Bellingham, WA 98225, and modified.

7. Apparatus for Method A

7.1 *Incubator*—capable of maintaining 37±0.5°C.

7.2 *Incubator*—capable of maintaining 42°C.

7.3 *Inoculating Loops*—3-mm.

7.4 *Bunsen Burner.*

7.5 *Pipettes*—sterile, wide-mouth 1-mL and 10-mL.

7.6 *Test Tube Racks.*

7.7 *Glassware*—autoclavable.

7.8 *Stomacher and stomacher bag*, (e.g., at least Model 400C, Seward Medical).

7.9 Pipettes—disposable, sterile, 1 and 10 mL.

7.10 *Culture Tubes.*

8. Reagents, Materials and Media for Method B

8.1 *Detection:*

8.1.1 *1-2 Test Kit*™—(Biocontrol Systems Inc., Bellevue WA, 98005 1-800-245-0113)—Store at 4 - 8°C until expiration date.8.1.2 *Lactose Broth.* Suspend 13 g of lactose powder in 1 L of deionized water. Dispense 4 × 225 mL into bottles, autoclave 12 - 15 min at 121°C and cool. Store for up to one month at room temperature, (~25°C).

8.1.2 *Brilliant Green Bile Broth, 2%*—Suspend 40 g in 1 L of deionized water and warm slightly to dissolve completely. Dispense into test tubes, autoclave for 12 to 15 min at 121°C and cool quickly. Store up to 3 months at room temperature, (~25°C).

8.1.3 *Iodine-Iodide Solution*—Dissolve 6 g of iodine and 5 g of potassium iodide in 20 mL of deionized water. Store at room temperature, (~25°C).

8.1.4 *Tetrathionate Broth*—Suspend 4.6 g of the powder in 100 mL of deionized water and mix thoroughly. Heat with frequent agitation and boil for 1 min to completely dissolve the powder. Cool to below 45°C and aliquot 10 mL into a sterile glass container with screw cap lid for each sample. Add 0.1 mL brilliant green bile broth and 0.2 mL iodine-iodide to each container. Use on the day of preparation only.

8.1.5 *Hektoen Enteric Agar Plates*—Suspend 46 g of agar in 600 mL deionized water. Boil until mixture does not boil over, usually about 15 min. Cool to 55°C and pour same day. Store up to one month in refrigerator, (~4°C).

8.1.6 *API 20E*®—BioMerieux Inc. (cat #20 179, St. Louis, Missouri, 1-800-638-4835), store in refrigerator until expiration date.

8.1.7 *Salmonella O Polyvalent Antiserum*—BBL (cat #40 707), store in refrigerator until expiration date, (~4°C).

8.2 *Quantification:*

8.2.1 *Peptone Broth (0.1%)*—dissolve 2.0 g of the powder in 1 L purified water and mix thoroughly. Dispense in 225 mL aliquots and autoclave at 121°C for 15 min.

8.2.2 *Selenite F Broth (1×)*—prepare fresh daily following manufacturer's directions.

8.2.3 *Hektoen-Enteric Agar*—prepare according to manufacturer's directions.

8.2.4 *MacConkey Agar*—prepare according to manufacturer's directions.

8.2.5 *Urea Slants*—purchased.

8.2.6 *TSI Slants*—purchased.

8.2.7 *LIA Slants*—purchased.

8.2.8 *API 20E Strips.*

8.2.9 *Blood Agar Plates*—purchased.

8.2.10 *Salmonella O Polyvalent Antisera.*

9. Sample Enrichment for Method A

9.1 *Day One*—Aseptically place 20 g as received compost into a sterile stomacher bag. Bring the weight up to 200 g with approximately 180 mL Peptone Water to achieve a 1:10 dilution of the sample.

9.1.1 Homogenize using a Stomacher machine at 260 rpm for two min.

9.1.2 Incubate for 24±2 h at 37°C.

9.1.3 For each new batch of 1-2 Test Kits™, inoculate a known *Salmonella* species into 10 mL of Lactose Broth and incubate overnight at 37°C. This serves as the positive control.

9.1.4 Determine the moisture content on a parallel compost sample (Method 03.09-A).

9.2 *Day Two*—Remove samples from the 1:10 Peptone Water dilution of compost and mix well. Transfer 1 mL of this 1:10 dilution to 10 mL of Tetrathionate broth/Brilliant Green/Iodine solution and incubate at 37°C for 24±2 h. Repeat this procedure starting with the 10 mL Lactose broth culture of the positive control sample.

10. Detection Procedure for Method A

10.1 Remove a required number of 1-2 Test Kit™ units from the refrigerator for samples and controls. Label flat surface of white cap.

10.2 Position 1-2 Test Kit™ unit with black cap up and remove black cap. Using sterile forceps, remove chamber plug and discard. Pour out contents of chamber and add 1.5 mL Tetrathionate broth mixture. Replace black cap.

10.3 Position 1-2 Test Kit™ unit with white cap up, and remove white cap. Snip off tip of Gel Void Former with shears and discard tip. The cut should be made at a point where tip meets base of Gel Void Former.

10.4 Add 1 drop (~1 mL) of Reagent #2 *(Antibody Preparation)* to Gel Void in motility chamber. Replace white cap. One drop of Reagent #2 should uniformly fill lower two-thirds of Gel Void.

10.5 Place inoculated 1-2 Test Kit™ units in incubator with white cap up. Incubate units 14 to 30 h at 37°C.

10.6 With white cap up, hold 1-2 Test Kit™ unit close to a strong light. Carefully observe motility chamber gel from all sides by rotating 1-2 Test Kit™ unit back and forth through 360° in front of light source.

CAUTION—1-2 Test Kit™ units are not reusable. Inoculated units may contain pathogenic organisms and should be handled carefully. All caps must be kept tightly secured. After use all units must be autoclaved at 121°C for 30 min before disposal.

11. Interpretation for Method A

11.1 *Positive Result:*

11.1.1 A positive 1-2 Test is indicated by the presence of an ImmunoBand in the upper half of the motility chamber gel.

11.1.2 ImmunoBand is a white band that is U-shaped or meniscus-shaped. ImmunoBand is three-dimensional and is seen in all planes while rotating a 1-2 Test Kit™ unit with white cap up. Band may be fully formed or may be more distinct on one side of gel.

11.1.3 Positive test indicates that sample presumptively contains *Salmonella*.

11.1.4 Positive 1-2 Test Kit™ should be confirmed by the culture method outlined in step 12 Confirming Results for Method A.

11.2 *Negative Result:*

11.2.1 If no band is seen after incubating the test unit for at least 14 h, test is negative.

11.2.2 Negative units may show uniform turbidity throughout motility chamber as result of movement of bacteria in gel.

12. Confirming Results for Method A

12.1 Presence of ImmunoBand indicates a presumptive salmonellae-positive sample. Perform cultural confirmation by streaking a loopful of inoculum from the tetrathionate, brilliant-green-serine enrichment broth onto a prepared Hektoen Enteric (HE) agar plate. Incubate 24±2 h at 37°C. Pick colonies typical of or suspected as salmonellae and inoculate an API 20E as per manufacturer's instructions. Perform serological tests on isolates identified as salmonellae.

13. Quantification Procedure for Method A

13.1 Prepare 1× Selenite broth in amounts needed for the number of samples to be tested. Aliquot 10 mL of 1× Selenite broth into separate tubes. Do not autoclave. Make HE plates if needed.

13.1.1 Prepare a 1:10 solids:liquid slurry by placing 20 g wet weight sample into a stomacher bag and adding Peptone Waterto bring the weight up to 200 g (approximately 180 mL).

13.2 Homogenize using a Stomacher machine at 260 rpm for two min.

13.2.1 Prepare a series of 10-fold dilutions in 0.1% Peptone Water by delivering 1 mL of the previous dilution into each subsequent 9 mL Peptone Water dilution blank. The number of dilutions that need to be prepared depends on the expected cell density.

13.2.2 Transfer 1 mL aliquots from the Peptone Water dilution series tubes to 5 tubes of 1× Selenite broth for each dilution increment, typically for the 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions.

13.3 *Confirmation Test*

13.3.1 Subculture each tube onto ½ HE plate and incubate at 42°C for 18 to 24 h.

13.3.2 Pick colonies suspected as Salmonellae (black centered green colonies) to urea slants, TSI and/or LIA slants. Salmonellae will be urea negative, TSI K/A+, and LIA K/NC+.

13.3.3 Confirm suspected colonies with API 20E, and if identified as salmonellae, confirm with serology.

14. Calculations for Method A

14.1 *Most Probable Number technique*—Record the number of positive tubes in each dilution set; select the highest dilution that gives positive results in all tubes (even if a lower dilution gives negative results) plus the next two higher dilutions. For dilutions prepared on samples at as-received moisture, convert the test aliquot size from wet weight to dw basis, (Method 03.09-A), and compute the MPN g^{-1} dw using the *MPN Calculator,* available on-line at http://tmecc.org/mpn/.

14.1.1 The MPN g^{-1} dw is then multiplied by four and reported as MPN $(4 \cdot g)^{-1}$ dw basis, i.e., total solids basis, to meet reporting method called for in USEPA CFR40503.

Test Method: *Salmonella.* Enrichment and Quantification of Salmonellae in Compost							**Units:** MPN $(4 \cdot g)^{-1}$, dw basis	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	07.02-B	07.02-B		07.02-B			07.02-B	

07.02-B ENRICHMENT AND QUANTIFICATION OF SALMONELLAE IN COMPOST

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

SUBMITTED BY—Soil Microbial Systems Laboratory; USDA-ARS-BARC; Beltsville, MD 20705

15. Apparatus for Method B

15.1.1 *Incubator*—convection, capable of maintaining 37±0.5°C.

15.1.2 *Glassware*—autoclavable.

15.1.3 *Pipettes*—disposable, sterile, 1-mL and 10-mL.

15.1.4 *Stomacher bag*, (e.g., Model 400C, Seward Medical).

15.1.5 *Conical tube*—50-mL, (e.g., Bluemax, Corning).

15.1.6 *Vortex mixer*

15.1.7 *Spiral-Plating Machine*—Optional, (e.g. Spiral Biotech).

16. Reagents, Materials and Media for Method B

16.1 *Buffered Peptone Water*, (e.g., Becton Dickinson).

16.2 *Tetrathionate Broth*, (e.g., TT-Hajna formulation).

16.3 *Xylose-Lysine Tergitol 4 agar*, (e.g., XLT4, Becton Dickinson).

16.4 *Culture Tubes*—screw top, 16- × 150-mm, (e.g., Fisherbrand).

17. Enrichment Procedure for Method B

17.1 Weigh a 25 g sample (as-received basis) directly into a sterile stomacher bag.

17.2 Add 225 mL of Buffered Peptone Water and blend at 260 rpm for 60 sec.

17.3 Place bag into 37ºC incubator for 18 to 24 h.

17.4 Homogenize bag manually, gently shake and massage.

17.5 Aseptically transfer 2.5 mL homogenate into a sterile 50-mL conical tube.

17.6 Add 22.5 mL Tetrathionate Broth (Hajna formulation) and vortex for 10 sec.

17.7 Place tube into a 35ºC incubator for 18 to 24 h.

17.8 Vortex the tube for 5 to 10 sec, and aseptically transfer two loops of broth mix onto XLT4 agar. Streak for isolation.

17.9 Incubate XLT4 plates for 24 h at 35ºC. If no black colonies are seen, the plates are then incubated for an additional 24 h (total of 48 h).

17.9.1 *Presumptive Positive*—All red colonies, red colonies with black centers, and black colonies are considered presumptive positive salmonellae.

17.10 Pick three presumptive positive colonies from the XLT4 plates and perform the biochemical and serological confirmation procedure (Method 07.02-C).

18. Quantification Procedure for Method B

18.1 Determine the moisture content on a parallel compost sample (Method 03.09-A).

18.2 *Most Probable Number System*

18.2.1 Place 20 g compost into a sterile stomacher strainer bag. Bring the weight up to 200 g with approximately 180 mL Buffered Peptone Water (BPW). This prepares a 1:10 dilution of the sample.

18.2.2 Homogenize using a Stomacher machine at 260 rpm for two min.

18.2.3 Prepare 1:100 and 1:1000 dilutions of the original sample homogenate (1:10) by pipetting 1 mL of the 1:10 dilution into 9 mL BPW dilution blanks, three times in succession.

18.2.3.1 Aseptically transfer 1 mL of the 1:10 sample homogenate into each of three screw top culture tubes containing 9 mL sterile BPW.

18.2.3.2 Aseptically transfer 1 mL of the 1:100 dilution into each of three screw top culture tubes containing 9 mL sterile BPW.

18.2.3.3 Aseptically transfer 1 mL of the 1:1000 dilution into each of three screw top culture tubes containing 9 mL sterile BPW.

18.2.4 Incubate all 9 mL tubes in a 37ºC incubator for 18-24 hours.

18.2.5 Vortex each 9 mL culture tube and aseptically transfer 1 mL from each into a 9 mL culture tube containing Tetrathionate (TT-Hajna) broth.

18.2.6 Vortex and incubate the TT tubes overnight for 18 to 24 h at 37°C.

18.2.7 Vortex each tube. Transfer a loopful of enrichment from each tube onto a sterile, surface-dried XLT4 agar plate.

18.2.8 Incubate XLT4 overnight for 24 h at 37°C. Follow the Serological and Biochemical Confirmation protocol (Method 07.02-C) on three presumptive positive isolates from each plate. Calculate the MPN g^{-1} dw score as described in section 19.1.

18.3 *Spread Plating Technique:*

18.3.1 Aseptically prepare one XLT4 agar plate for use with each sample (if using a mechanical Spiral Plater), or five XLT4 plates for use with standard spread plating methods.

18.3.2 If using a spiral-plating machine plate 50:l from the 10^{-2} dilution used during the MPN protocol above. Incubate for 24 h and follow one of the serological and biochemical confirmation protocols in Method 07.02-C. If no presumptive positives are apparent, re-incubate for an additional 24 h. Calculate the cfu g^{-1} for all presumptive positive colonies using the method described in Step 19.2.

18.3.3 If using standard spread plating methods, plate 100:l onto individual XLT4 plates from each of the following dilutions: 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} onto individual XLT4 plates. Use only surface-dried XLT4 agar plates at room temperature. Incubate for 24 h and follow one of the serological and biochemical confirmation protocols (Method 07.02-C). Presumptive positive colonies on XLT4 should appear black or red with black centers. If no presumptive positives are apparent, re-incubate for an additional 24 h.

18.3.4 Quantify all presumptive salmonellae-positive colonies on the XLT4 spread or spiral plates using the method described in Step 19.3.

19. Calculations for Method B.

19.1 *Most Probable Number technique*—Record the number of positive tubes in each dilution set; select the highest dilution that gives positive results in all tubes (even if a lower dilution gives negative results) and the next two higher dilutions). For dilutions prepared with samples at as-received moisture, convert the test aliquot size from wet weight basis to dry weight basis, and compute the MPN g^{-1} dw using the *MPN Calculator*, available on-line at http://tmecc.org/mpn/.

19.1.1 *MPN Reporting*—The MPN g^{-1} dw is multiplied by four and reported as MPN $(4 \cdot g)^{-1}$ dw basis, i.e., total solids basis, to meet the reporting method called for in US EPA 40CFR 503.

19.2 *Spiral Plating technique*—Calculate the total presumptive salmonellae-positive colonies, cfu g^{-1} dw using the protocols included by the manufacturer for the model spiral-plater used.

19.3 *Standard Spread Plating technique*—count the presumptive salmonellae-positive colonies and calculate the cfu $(4 \cdot g)^{-1}$ dw basis using the following formula:

$$CFU = (C \div V) \times D \times TS \times 4 \qquad \textit{Equation 19.3}$$

where:

CFU = colony forming units per gram of sample; number of cells in original sample, cfu g^{-1} dw,

C = number of colonies of the target organism, (e.g., coliforms = pink or red colonies),

V = volume plated, mL, i.e., 100µL = 0.1 mL; 50 µL = 0.05 mL, etc.,

D = dilution factor, mL g^{-1}; (e.g., 1 ÷ dilution, i.e., 1 ÷ 10^{-2} = 10; 1 ÷ 10^{-4} = 1,000, etc.),

TS = total solids ratio = mass of oven dried aliquot ÷ mass of aliquot at as-received moisture, (refer to Method 03.09-A), and

4 = 4.0 g, multiplier needed to meet reporting method called for in US EPA 40CFR 503.

Test Method: *Salmonella.* Confirmation Protocols for Presumptive *Salmonella* Isolates							**Units:**	
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
	07.02-C	07.02-C		07.02-C			07.02-C	

07.02-C CONFIRMATION PROTOCOLS FOR PRESUMPTIVE *SALMONELLA* ISOLATES

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

SUBMITTED BY—Soil Microbial Systems Laboratory; USDA-ARS-BARC; Beltsville, MD 20705

20. Apparatus for Method C

20.1 *Incubator*—convection, capable of maintaining 37±0.5°C.

20.2 *Inoculation Loops*

21. Reagents, Materials and Media for Method C

21.1 *Biochemical/Serological Procedure*

21.1.1 Prepare two-biochemical media: Triple Sugar Iron Agar (e.g., TSI, Becton Dickinson) and Motility Indole Lysine Agar (e.g., MIL, Becton Dickinson). Both can be prepared according to the manufacturer's instructions, and in screw-top culture tubes. TSI should be made into slants with a generous slant on the top of the tube. MIL should be made into agar deeps (enhancing oxygen deprivation). Prepare each medium according to the manufacturer's instructions, (e.g., Becton Dickinson).

21.1.2 Kovac's reagent, (e.g., Becton Dickinson).

21.1.3 poly-O *Salmonella* antiserum, (e.g., Becton Dickinson).

21.2 *Colony Lift Immunoassay Procedure (CLI)*—Commercially available kit from Kirkegaard and Perry Laboratories (KPL, Gaithersburg, MD 20879, 1-800-638-3167).

22. *Option One*—Biochemical / Serological Procedure for Method C

22.1 Pick a colony of presumptive salmonellae from the XLT4 plate using sterile needle and inoculate the medium in the following manner: Inoculate TSI agar by first streaking the slant, then stabbing into the solid non-slant end of the agar, on the deep end of the tube (the area known as the butt of the agar slant). With the same needle, stab the butt of the MIL agar deep twice.

22.2 Incubate both TSI and MIL tubes for 18hr at 35°C.

22.3 Observe coloration of the medium. Presumptive salmonellae on TSI will have an acid butt (yellow) and basic slant (red). Many will also produce hydrogen sulfide, which will be present as a black coloration in the butt. This color will often mask the yellow coloration in the butt. Presumptive salmonellae on MIL will exhibit a purple coloration on both top and bottom of the tube, and salmonellae will also have swarmed throughout the medium (the stab-line should not be visible). Add two drops of Kovac's reagent. Salmonellae do not produce indole, as exhibited by a red-band formation on the surface of the medium after the addition Kovac's reagent.

22.4 For each presumptive positive isolate, perform serology using a slide-agglutination technique by using the cell material from the TSI slant, and

22.5 Perform serology using a slide-agglutination technique. This is performed using poly-O *Salmonella* antiserum as well as specific antiserum groups A-E, and is performed according to the manufacturers instructions.

NOTE 1B—Salmonellae remain **presumptive** positive until the antiserum is used to confirm the isolates.

23. *Option Two*—Colony Lift Immunoassay Procedure for Method C

23.1 The Colony Lift Immunoassay (CLI) is a rapid and sensitive immunological method for detecting a broad range of *Salmonella* serotypes in mixed culture. The kit allows for simultaneous screening of multiple colonies on an agar plate through a single 20-minute assay. The peroxidase-labeled anti-broad spectrum *Salmonella* antibody used in the kit is polyvalent for all *Salmonella* group O antigens.

23.2 To perform the CLI procedure, follow the manufacturer's instructions. Basically, an impression of the colonies (grown on the spiral or spread plate) is made by lightly overlaying a protein-binding membrane onto the XLT4 agar surface. Bacterial antigen from each of the colonies binds to the membrane surface. The membrane is then incubated with the peroxidase-labeled conjugate (*Salmonella* antibody), which binds to the antigen that is firmly attached to the protein-binding membrane. After the membrane is washed to remove excess conjugate, a sensitive peroxidase substrate is added which deposits

a blue color to the sites on the membrane where conjugate is bound to the *Salmonella* antigen.

24. Interpretation of Results for Method C

24.1 The result is a membrane containing a mirror image of colonies that were originally 'lifted' from the XLT4 agar plate. Imprints that were lifted from colonies that contain *Salmonella spp*. antigen will turn blue on the membrane, and non-*Salmonellae* antigen will remain colorless. This testing procedure does not harm the original colonies on the XLT4 plate, which can be isolated and stored for future characterization. The Colony Lift Immunoassay is a fast, reliable and sensitive assay for the confirmation of presumptive salmonella*e* grown on XLT4 agar plates, as well as a variety of other selective and non-selective media.

07.02 METHODS SUMMARY

25. Report

25.1 *Method 07.02-A 1-2 Detection Test and Quantification Procedure for Salmonella in Compost*—Report results as either positive or negative. Report confirmation test and include result. Report MPN $(4 \cdot g)^{-1}$, *Note:* dw basis.

25.2 *Method 07.02-B Enrichment and Quantification of Salmonellae in Compost*—Report results as either positive or negative. Report confirmation test and include result. Report MPN $(4 \cdot g)^{-1}$, *Note:* dw basis.

25.3 *Method 07.02-C Confirmation Protocols for Presumptive Salmonella Isolates*—Report confirmation test and include result.

26. Precision and Bias

26.1 The precision and bias of the tests listed below have not been determined. Data are being sought for use in developing a precision and bias statement.

26.1.1 *Method 07.02-A 1-2 Detection Test and Quantification Procedure for Salmonella in Compost*

26.1.2 *Method 07.02-B Enrichment and Quantification of Salmonellae in Compost*

26.1.3 *Method 07.02-C Confirmation Protocols for Presumptive Salmonella Isolates*

27. Keywords

27.1 bacteria; pathogen; Salmonella; compost; detection; quantification

Test Method: Enterococci. One Method						**Units:** MPN g^{-1}, dw basis		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
				07.03-A		07.03-A	07.03-A	

07.03 ENTEROCOCCI

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org/addenda.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This test covers the determinations for fecal streptococci and enterococci in compost .

1.1.1 *Method 07.03-A Enterococcus.*

1.2 Values stated in SI units are to be regarded as the standard. Values given in parentheses are provided for information only.

2. Referenced Documents

2.1 *TMECC:*

Method 02.02-B Sample Sieving for Aggregate Size Classification.

Method 03.09-A Total Solids and Moisture at 70±5°C.

Section 07.01 Coliform Bacteria.

Section 07.02 *Salmonella*.

2.2 *Standard Methods for the Examination of Water and Wastewater:*

SM 9230 Fecal Streptococcus and Enterococcus Group.

SM 9260D Detection of Pathogenic Bacteria, pp. 9-91.

FDA Bacteriological Analytical Manual, 8th Edition (Revision A), 1998, Appendix 2.07.

Official Methods of Analysis of AOAC International, 14th Edition, Table 46:01, 1984, p.943.

3. Terminology

3.1 *Enterococci, n pl*—Gram-positive bacteria that were formerly classified as Group 'D' streptococci. In 1984, several members of the Group 'D' streptococci were reclassified as a new genus, *Enterococcus*, for clinical reasons. Enterococci now represent a small portion of organisms that constitute the fecal streptococci. Enterococci may be used as 'indicator organisms' for fecal contamination in the same way that Group 'D' streptococci were. Enterococci, or 'enteric cocci', are commonly found in fecal material of humans and a variety of animals. These organisms can survive harsh conditions for longer periods of time in the environment than either total or fecal coliforms, *E. coli* or salmonellae. For example, enterococcus can grow in the presence of 6.5% sodium chloride and at 45°C, and also survive at temperatures as high as 60°C.

3.2 *indicator organisms, n*—Microbes that are generally non-pathogenic, but co-exist in habitats with pathogens. Detection and quantification of an indicator organism in a sample is presumptive evidence that pathogens may also be present in the habitat from which the sample was obtained. Detection and quantification of indicator organisms is often much simpler and less costly than detecting/quantifying specific pathogens.

4. Summary of Test Method

4.1 *Method 07.03-A Enterococcus*—Methods described in the Standard Methods for the Examination of Water and Wastewater were adapted for the quantification of *Enterococcus* in compost. The fecal streptococci/enterococci quantification method combines traditional spread plating techniques with an MPN system for more rapid and sensitive quantification. These methods may be easily performed simultaneously with the salmonella and coliform bacteria protocols, (*c.f.*, Methods 07.01 and 07.02). Modified *Enterococcus* agar (mEnt) is a presumptive positive medium for *Enterococci*, and the plates may be counted after 48 h of incubation at 35ºC.

5. Significance and Use

5.1 *Method 07.03-A Enterococcus*—Enterococci are indicator organisms of fecal contamination. They colonize the colon of humans and animals without infection and are shed in the feces. Enterococcus has become well recognized in the 1990's as the fourth leading cause of hospital-acquired infections and for its ability to cause life-threatening infections, especially in patients with urinary or intravascular catheters, with

intra-abdominal abscesses, and in patients that have received broad-spectrum antibiotics. The most significant current concern with *Enterococcus* is its propensity for developing resistance to conventional antibiotics, and the transmission of this resistance to other (more virulent) organisms via mobile genetic elements known as plasmids.

6. Interference and Limitations

6.1 *Method 07.03-A Enterococcus*—Most Probable Number (MPN) methods have several limitations including the requirement of time, effort and equipment required to handle large quantities of materials per sample. Direct plating onto very selective and differential media, i.e., Modified Enterococcus agar, has been reported to be inefficient in growing organisms that have been injured or are described as being viable but not culturable. The simultaneous strategy of using both a limited MPN and spread plates help avoid the massive equipment required for the MPNs while eliminating the low sensitivity and cultivability problems prevalent with spread plates.

7. Sample Handling

7.1 Samples at as-received moisture content are used for this test. Moisture analysis of a parallel sample aliquot must be conducted so that data can be calculated and reported on a dry weight basis. If delays in analysis are anticipated, store compost samples in sealed containers at approximately 4°C. Large compost samples must be homogenized and mixed thoroughly before the microbial test sample aliquots analysis are collected. Thorough mixing of samples minimizes the heterogeneous distribution of microbes.

Test Method: Enterococci. *Enterococcus*						**Units:** MPN g^{-1}, dw basis		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	Safety Standards	Market Attributes
				07.03-A		07.03-A	07.03-A	

07.03-A *ENTEROCOCCUS*

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

8. Apparatus for Method A

8.1 *Most Probable Number Technique:*

8.1.1 *culture tubes*—16-mm × 150-mm, screw-top tubes, (e.g., Fisherbrand).

8.1.2 *dilution tubes*

8.1.3 *incubator*—set at 35 to 37ºC.

8.1.4 *inverted gas tubes*—6-mm × 50-mm, (e.g., Fisherbrand).

8.1.5 *strainer bag*—sterile stomacher bag, (e.g., Stomacher Model 400C, Seward Medical).

8.2 *Spread Plating Technique:*

8.2.1 *spiral-plating machine*—optional, (e.g., Spiral Biotech).

9. Reagents and Materials for Method A

9.1 *Most Probable Number Technique:*

9.1.1 *Buffered Peptone Water*—BPW, (e.g., Difco).

9.1.2 *Modified Enterococcus Agar*—mEnt, (e.g., Difco).

9.1.3 *Brain Heart Infusion*—BHI, modified broth containing additional sodium chloride (total 6.5% NaCl).

9.1.4 *Azide Dextrose Broth*—AD Broth, (e.g., Difco).

9.2 *Spread Plating:*

9.2.1 *ethanol*—70% for sterilization.

9.2.2 *Modified Enterococcus Agar* –mEnt, (e.g.,Difco).

10. Procedure for Method A

NOTE 1A—This method can be performed simultaneously with the coliform (TMECC 07.01) and salmonella (TMECC 07.02-B) protocols.

10.1 *Prepare 10^{-1} Homogenate*—Place 20 g of compost into sterile stomacher bag. Bring weight up to 200 g with the addition of 180ml buffered peptone water (BPW) for a 1:10 dilution.

10.2 Homogenize by stomaching on a Seward 400C Stomacher machine for 2 min at 260 rpm. (Note: A sterile laboratory blender may also be used, on 'high' setting for one minute)

10.3 *Most Probable Number (MPN) Technique:*

10.3.1 Prepare three additional dilutions by performing three 1:10 serial dilutions in sterile BPW containing, respectively, 10^{-2}, 10^{-3} and 10^{-4} dilutions of the original compost sample. This can be done by adding 1 mL of the original sample homogenate (10^{-1}) to 9 mL BPW, vortexing for 5-10 sec, and continuing this dilution scheme two more times. These will be used to inoculate the MPN tubes described below.

10.3.2 Prepare nine screw-top culture tubes, each containing 9 mL sterile Azide Dextrose broth (AD).

10.3.2.1 Aseptically transfer 1 mL of the 1:10 (10^{-1}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile AD.

10.3.2.2 Aseptically transfer 1 mL of the 1:100 (10^{-2}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile AD.

10.3.2.3 Aseptically transfer 1 mL of the 1:1000 (10^{-3}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile AD.

10.3.3 Incubate tubes for 24 h in a 37ºC incubator.

10.3.4 Observe the AD tubes for presence of growth (turbidity).Vortex each tube and streak (one loopful) onto the surface of modified Enterococcus Agar plate (mEnt). Simultaneously pipet 20-40µL from each tube into 9mL Brain Heart Infusion Broth containing 6.5% Sodium Chloride (NaCl).

10.3.5 Incubate mEnt plates for 24 – 48 h in a 37ºC Incubator. Record the number of plates in each dilution set that are positive for growth. This number will be used to calculate the MPN g^{-1} (Most Probable Number per gram sample) for fecal streptococci, which includes enterococci.

10.3.6 Incubate the BHI + 6.5% NaCl tubes in a 37ºC for 24 h. Record the number of tubes in each dilution set that are positive for growth. This number will be used to calculate the MPN g^{-1} dw (Most

Probable Number per gram sample, dw basis) for Enterococci.

10.4 *Spread Plating technique:*

10.4.1 Prepare one modified Enterococcus Agar (mEnt) plate if using an automated spiral-plating machine, or six plates if using standard spread plating techniques. Air dry the surface of the plates by maintaining them at room temperature for one day, or place into a laminar flow hood for 10 min with the lids removed.

10.4.2 If using an automated spiral-plating machine, plate 50 µL from the 10^{-2} dilution used during the MPN protocol above. Incubate for 18 to 24 h at 37ºC.

10.4.3 If using standard spread plate methods, place 100 µL from the 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions onto the surfaces of a different mEnt plates. Spread the liquid evenly onto the surface of each agar plate using a glass 'hockey-stick' that has been dipped in 70% ethanol and briefly passed under a flame. The sample spreading procedure is facilitated by use of a rotating Petri-dish holder. Incubate all plates at 37ºC for 24 h to 48 h.

10.4.4 Observe the agar surface for growth of all colonies, which may appear red, purple or absent of color. All surface growth is considered to be enterococcus.

11. Calculations for Method A

11.1 *Most Probable Number Technique*—Record the number of positive tubes in each dilution set. Select the highest dilution that gives positive results in all tubes (even if a lower dilution gives negative results), plus the next two higher dilutions. For dilutions prepared with 'as received' samples, i.e., wet weight basis, convert the test aliquot size from wet weight basis to dry weight basis by multiplying each dilution times the total solids ratio (refer to Method 03.09-A), and compute the MPN using the *MPN Calculator,* available on-line at http://tmecc.org/mpn/.

11.2 Quantify the enterococci as colony-forming units (cfu g^{-1} dw) using the protocols included by the manufacturer of the spiral-plating equipment used. If using standard spread plating techniques, count all colonies and perform the calculation using the following formula:

$$CFU = (C \div V) \times D \times TS \qquad \textit{Equation 11.2}$$

where:

CFU = colony forming units per gram of sample; number of cells in original sample, cfu g^{-1} dw,

C = number of colonies of the target organism, (e.g., coliforms = pink or red colonies),

V = volume plated, mL, i.e., 100µL = 0.1 mL; 50 µL = 0.05 mL, etc.,

D = dilution factor, mL g^{-1}; (e.g., 1 ÷ dilution, i.e., 1 ÷ 10^{-2} = 10; 1 ÷ 10^{-4} = 1,000, etc.), and

TS = total solids ratio = mass of oven dried aliquot ÷ mass of aliquot at as-received moisture, (refer to Method 03.09-A).

07.03 METHODS SUMMARY

12. Report

12.1 *Method 07.03-A Enterococcus*—Depending on the concentration of enterococci in the sample, results will be reported in either cfu g^{-1} dw (spread plating technique) or MPN g^{-1} dw (Most Probable Number technique); or both. Results should be reported on a dry weight (dw) basis for all quantification tests.

13. Precision and Bias

13.1 *Method 07.03-A Enterococcus*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

14. Keywords

14.1 enterococci; fecal streptococci; most probable number; spread plating; detection; quantification; pathogen; compost

Test Method: Parasitic Helminths. One Method.						**Units:** method specific		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
				07.04-A		07.04-A	07.04-A	

07.04 PARASITIC HELMINTHS

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(2) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://www.tmecc.org/addenda.

(3) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Scope

1.1 This section describes the detection, enumeration, and determination of viability of parasitic helminths in compost.

1.1.1 *Method 07.04-A Viable Ascaris Ova in Compost*—These pathogenic intestinal helminths occur in domestic animals and humans. The environment may become contaminated through direct deposit of human or animal feces or through sewage and wastewater discharges to receiving waters. Ingestion of water containing infective *Ascaris* ova may cause disease..

1.2 This test method is for wastewater, sludge, and compost. It is the user's responsibility to ensure the validity of this test method for untested matrices.

2. Referenced Documents

2.1 *TMECC:*

Method 03.09-A Total Solids and Moisture at 70±5°C.

2.2 *Other Sources:*

Geenen, P.L., J. Bresciani, Jeap Bees, A. Pedersen. Lis Eriksen, H.P. Fagerholm, and P. Nansen. 1999. The Morphogenesis of *Ascaris suum* to the infective third-stage larvae within the egg, J. Parasitol. 65:616-622.

Reimers, R.S., M.D. Little, T.G. Akers. W.D. Henriques, R.C. Badeaux, D.B. McDonnel, and K.K. Mbela. 1989. Persistance of pathogens in lagoon-stored sludge. Cooperative Agreement N. 810289. U.S. Environmental Protection Agency. EPA1600/2-89/015.

3. Terminology

3.1 *Ascaris*—A pale colored nematode genus with a round body that is tapered at both ends; parasite of mammals; lives in the intestines of mammals; produces enzyme inhibitor's that protect it from host's digestive enzymes.

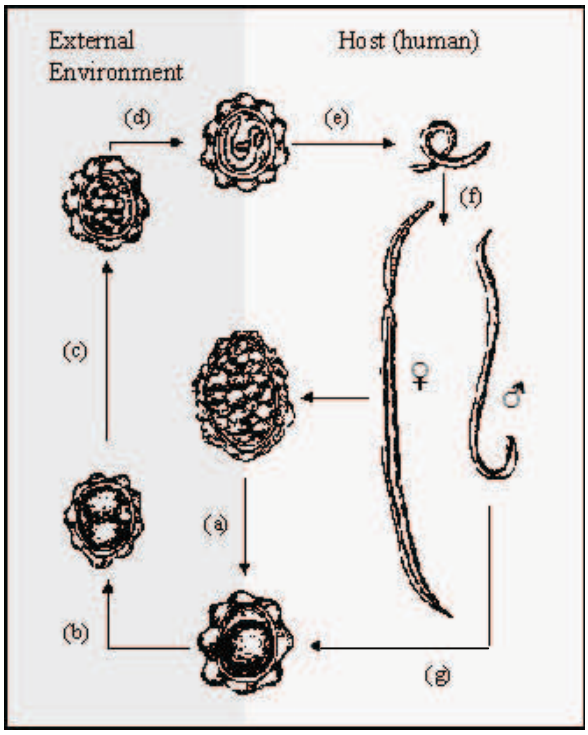

Fig 07.04-1 Life Cycle of *Ascaris* sp. (a) the unfertilized egg; (b) eggs in faeces, diagnostic stage; (c) embryonation; (d) embryonated egg, infective stage; (e) ingestion by host, larvae hatch in intestine; (f) circulation through lungs, trachea and pharynx to small intestine; (g) fertilized egg, adults in lumen of small intestine.

3.2 The normal nematode life cycle consists of the egg, four larval stages and an adult. Larvae are similar in appearance to the adults, i.e., typically worm-like in appearance.

3.3 Molting (ecdysis) of the outer layer (cuticle) takes place after each larval stage. Molting consists of two distinct processes, the deposition of the new cuticle and the shedding of the old one or exsheathment. The cuticle appears to be produced continuously, even throughout adult life.

3.4 A molted cuticle that still encapsulates a larva is called a sheath.

3.5 *Ascarid* egg shells are commonly comprised of layers. The outer tanned, bumpy layer is referred to as the mammillated layer and is useful in identifying *Ascaris* eggs. The mammillated layer is sometimes absent. Eggs that do not possess the mammillated layer are referred to as decorticated eggs.

3.6 A potentially infective *Ascaris* egg contains a third stage larvae (L3) encased in a the sheath of the first larval stage (L1, see Fig 07.04-A2).

4. Summary of Test Methods

4.1 *Method 07.04-A Viable Ascaris Ova in Compost*—This method is used to concentrate pathogenic *Ascaris* ova from wastewater, sludge and compost. Samples are processed by blending with buffered water containing a surfactant. The blend is screened to remove large particulates. The solids in the screened portion are allowed to settle out and the supernatant is decanted. The sediment is subjected to density gradient centrifugation using magnesium sulfate (specific gravity 1.20). This flotation procedure yields a layer likely to contain *Ascaris* and some other parasitic ova if present in the sample. Small particulates are removed by a second screening on a small mesh size screen. Proteinaceous material is removed using an acid-alcohol/ethyl acetate extraction step. The resulting concentrate is incubated at 26°C until control *Ascaris* eggs are fully embryonated. The concentrate is then microscopically examined for the categories of *Ascaris* ova on a Sedgwick-Rafter counting chamber.

5. Significance and Use

5.1 *Method 07.04-A Viable Ascaris Ova in Compost*—Determinations from this test provide quantitative indications of the level of *Ascaris* ova contamination of wastewater, sludge, or compost and may be useful in evaluating the effectiveness of treatment.

6. Interference and Limitations

6.1 Freezing of samples will interfere with the buoyant density of helminth ova and decrease the recovery of ova.

6.2 Solids and debris present in the sludge being viewed with the microscope were found to impair ones ability to count. Dilution of raw sludge and digested sludge, however, with phosphate-buffered water prior to analyzing them significantly improved the number of ova that could be counted. Raw sludges were diluted by a factor of 20 and digested sludges by a factor of five. QA/QC procedures were followed to validate this procedure.

6.3 *Method 07.04-A Viable Ascaris Ova in Compost*—This test method will not identify the species of *Ascaris* detected nor the host of origin.

7. Sampling Handling

7.1 *Method 07.04-A Viable Ascaris Ova in Compost:*

7.1.1 Collect 1,000 cm^3 of compost in accordance with TMECC 02.01-B.

7.1.2 Place the sample container(s) on wet ice or around chemical ice and ship back to the laboratory for analysis within 24 hours of collection.

7.1.3 Store the samples in the laboratory refrigerated at 4°C. Do not freeze the samples during transport or storage.

Test Method: Parasitic Helminths. Viable *Ascaris* Ova in Compost.						**Units:** viable ova $(4 \cdot g)^{-1}$, dw basis		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
				07.04-A		07.04-A	07.04-A	

07.04-A VIABLE *ASCARIS* OVA IN COMPOST

LOOK—Interference and Limitations, and Sampling Handling issues are presented as part of the introduction to this section.

ADAPTED FROM—US EPA Document 625r92013. Control of Pathogens and Vector Attraction in Sewage Sludge, Appendix I. Test Method for Detecting, Enumerating, and Determining the Viability of *Ascaris* Ova in Sludge. Frank Schaefer - Biohazard Assessment Research Branch, National Exposure Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, USA 45268.

8. Apparatus for Method A

8.1 *Microscope*—A good light microscope equipped with bright field, and preferably with phase contrast and/or differential contrast optics including objectives ranging in magnification from 10× to 45×.

8.2 *Sedgwick-Rafter cell.*

8.3 *Beakers*—2-L, (e.g., Pyrex or equivalent).

8.4 *Erlenmeyer flask*—500-mL.

8.5 *Centrifuge*—to sustain forces of at least 660 *g* with swinging bucket rotor to hold centrifuge glass or plastic conical bottles and tubes:

8.5.1 100- or 250-mL conical bottles, and

8.5.2 15-mL centrifuge tubes.

8.6 *Sieves*—12-cm (5-in.) diameter, (e.g., Tyler):

8.6.1 20-mesh or 50-mesh, stainless steel, and

8.6.2 400-mesh, stainless steel.

8.6.3 Large plastic funnel to support the sieve.

8.7 *Spatula*—Teflon, or equal.

8.8 *Incubator*—set at 26°C.

8.9 *Test tube racks*—to accommodate centrifuge tubes*:*

8.9.1 large, for 100- or 250-mL tubes.

8.9.2 small, for 15-mL tubes.

8.10 *Centrifuge tubes (Coat with organosilane):*

8.10.1 100-mL or 250-mL.

8.10.2 15-mL, conical.

8.11 *Applicator sticks*—wooden.

8.12 *Pasteur pipettes*.

8.13 *Vacuum aspiration apparatus*—vacuum source; vacuum flask, 2-L or larger; and stopper to fit vacuum flask, fitted with a glass or metal tubing as a connector for ¼ in. Tygon tubing.

8.14 *Wash bottles*—500-mL, label *water.*

8.15 *Spray bottles*—500-mL (16 oz), two.

8.15.1 Label one *water*.

8.15.2 Label one *1% 7×*.

9. Reagents and Materials for Method A

9.1 *Water*—Type I.

9.2 *Phosphate buffered water*—One L = 34.0 g KH_2PO_4, pH adjusted to 7.2 + 0.5 with 1 *N* NaOH.

9.3 *Laboratory detergent*—1% (v/v) 7× Limbro laboratory detergent (ICN Biochemicals; Aurora, OH). 1 L = 999 mL phosphate-buffered water, 1 mL 7× Limbro - adjust pH to 7.2 + 0.1 with 1 *N* NaOH.

9.4 *Magnesium sulfate*—with specific gravity of 1.20. One L = 215.2 g $MgSO_4$ – verify specific gravity with a hydrometer; adjust as necessary to reach 1.20).

9.5 *Organosilane*—For coating glassware (e.g., Sigmacote, Sigma Chemical Company, St. Louis, MO). Coat all glassware according to manufacturer's instructions.

9.6 *Positive Control*—Harvest fecally derived ova for positive control, purified from *Ascaris* infected pig fecal material.

10. Procedure for Method A

10.1 The moisture content of the sample is determined on a separate portion of the sample; it is used to calculate number of ova per gram dry weight can be determined (refer to TMECC 07.09 Total Solids and Moisture). The concentration of ova in liquid sludge samples may be expressed as ova per unit volume.

10.2 *Initial preparation:*

10.2.1 *Dry or thick samples*—Transfer 300 g, dry-weight equivalents, of compost at as-received moisture and at least 500 mL of water in a beaker and let soak overnight at 4°C to 10°C. Transfer to a blender and blend at high for one minute. Divide sample into four beakers.

10.2.1.1 Determine the dry-weight equivalent aliquot size as follows, g:

$$A = B \div [C \times 0.01] \qquad \textit{Equation 10.2.1.1}$$

where:

A = mass of as-received moist compost aliquot, g
B = dry-weight equivalent of sample, e.g., 300 g,
C = sample total solids content, % wet weight basis by Method 03.09-A, and
0.01 = factor to convert from percentage to fraction, unitless.

NOTE 1A—The optimal ratio of water to compost for this step is not adequately defined. Further experimentation to identify an appropriate ratio is strongly recommended.

10.2.2 *Liquid samples*—Measure 1,000 mL or more (estimated to contain at least 50 g dry solids) of liquid sample. Place one half of the sample in a blender. Add about 200 mL water. Blend at high speed for one minute and transfer to a beaker. Repeat for the other half of the sample.

10.3 Pour the homogenized sample into a 1,000 mL tall form beaker and using a wash bottle, thoroughly (analytically) rinse the blender container into the beaker. Add 1% 7× surfactant to reach 900 mL final volume.

10.4 Allow the sample to settle four hours or overnight at 4°C to 10°C. Stir occasionally with a wooden applicator as needed to ensure that material floating on the surface settles. Additional 1% 7× may be added, and the mixture stirred if necessary.

10.5 After settling, vacuum aspirate supernatant to just above the layer of solids. Transfer sediment to blender and add water to 500 mL, blend again for one minute at high speed.

10.6 Transfer to beaker, rinsing blender and add 1% 7× to reach 900 mL. Allow to settle for two hours at 4°C to 10°C, vacuum aspirate supernatant to just above the layer of solids.

10.7 Add 300 mL 1% 7× and stir for five minutes on a magnetic stirrer.

10.8 Strain homogenized sample through a 20-mesh or 50-mesh sieve placed in a funnel over a tall beaker. Wash the sample through a sieve with a spray of 1% 7× from a spray bottle.

10.9 Add 1% 7× to 900 mL final volume and allow to settle for two hours at 4°C to 10°C.

10.10 Vacuum aspirate supernatant to just above layer of solids. Mix sediment and distribute equally to 50 mL graduated conical centrifuge tubes. Thoroughly wash any sediment from the beaker into tubes using water from a wash bottle. Bring volume in tubes up to 50 mL with water.

10.11 Centrifuge for ten minutes at 1,000 *g*. Vacuum aspirate supernatant from each tube down to just above the level of sediment.

NOTE A2—The packed sediment in each tube should not exceed five mL. If it exceeds this volume, add water and distribute the sediment evenly among additional tubes, repeat centrifugation, and vacuum aspirate supernatant.

10.12 Add 10 to 15 mL of $MgSO_4$ solution (specific gravity 1.20) to each tube, cap and mix for 15 sec to 20 sec on a vortex mixer.

NOTE A3—Use capped tubes to avoid splashing of mixture from the tube while vortex mixing.

10.13 Add additional $MgSO_4$ solution (specific gravity 1.20) to each tube to bring volume to 50 mL. Centrifuge for five to ten min at 800 *g* to 1,000 *g*. DO NOT USE BRAKE.

10.14 Allow the centrifuge to coast to a stop without the brake. Pour the top 25 mL to 35 mL of supernatant from each tube through a 400-mesh sieve supported in a funnel over a tall beaker.

10.15 Using a water spray bottle, wash excessive flotation fluid and fine particles through sieve.

10.16 Rinse sediment collected on the sieve into a 100-mL beaker by directing the stream of water from the wash bottle onto the upper surface of the sieve.

10.17 After thoroughly washing the sediment from the sieve, transfer the suspension to the required number of 15-mL centrifuge tubes, taking care to rinse the beaker into the tubes. Usually one beaker makes one tube.

10.18 Centrifuge the tubes for three minutes at 800 *g*, then discard the supernatant.

10.19 If more than one tube has been used for the sample, transfer the sediment to a single tube, fill with water, and repeat centrifugation.

10.20 Aspirate the supernatant above the solids.

10.21 Re-suspend the solids in 4 mL 0.1 *N* H_2SO_4 and pour into a 20.0-mL polyethylene scintillation vial or equivalent with loose caps.

10.22 Before incubating vials, mark the liquid level in each vial with a felt tip pen. Incubate sample vials, along with control vials containing *Ascaris* ova mixed with 4 mL 0.1 *N* H_2SO_4 at 26°C for three to four weeks. Every day or so, check the liquid level in each vial. Add reagent grade water up to the initial liquid level line as needed to compensate for evaporation. After 18 days, suspend, by inversion and sample small aliquots of the control cultures once every two to three days. When the majority of the control *Ascaris* ova are fully embryonated, samples are ready to be examined.

10.23 Examine the concentrates microscopically using a Sedgwick-Rafter cell to enumerate the detected

ova. Classify the ova as either unembryonated (Fig 07.04-A1), or embryonated to the first (L1, Fig 07.04-A2), second (L2) or third (L3) larval stage. In some embryonated *Ascaris* ova the larva may be observed to move.

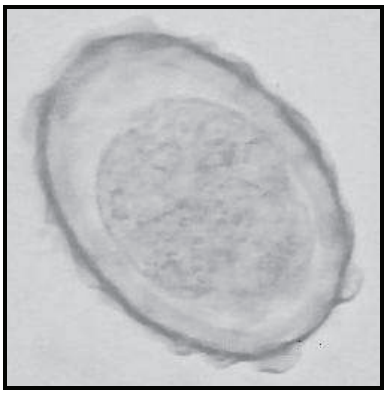

Fig 07.04-A1 *Ascaris lumbricoides* fertilized egg covered with a thick shell that appears mammillated; size approx. 65 µm.

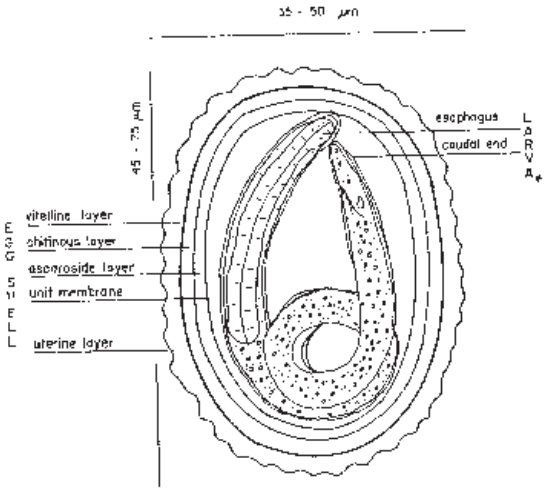

Fig 07.04-A2 Line drawing of an embryonated *Ascaris* egg containing the third stage larva, L3.

11. Calculation for Method A

11.1 Calculate the categories of ova per four grams of compost, dry weight:

$$Ova = [4 \times NO \times CV \times FV] \div [SP \times TS] \quad \textit{Equation 11.1}$$

where:

Ova = number of Viable Ova per four grams of compost, dw basis

NO = number of ova counted,

CV = chamber volume, 1 mL,

FV = final volume, mL,

SP = amount of sample processed, g (as-received basis for solid samples) or mL (liquid samples), and

TS = total solids content of sample, unitless ratio, refer to TMECC 03.09 Total Solids and Moisture.

07.04 METHODS SUMMARY

12. Report

12.1 *Method 07.04-A Viable Ascaris Ova in Compost*—Report the results as the total number of *Ascaris* ova, number of unembryonated *Ascaris* ova, number of first, second, or third stage larvae (L1, L2, or L3); reported as number of *Ascaris* ova and number of various larval *Ascaris* per four grams of compost, dry weight basis.

13. Precision and Bias

13.1 *Method 07.04-A Viable Ascaris Ova in Compost*—The precision and bias of this test have not been determined. Data are being sought for use in developing a precision and bias statement.

14. Keywords

14.1 *Ascaris,* ova, embryonation, viability assay, helminth.

APPENDIX TO 07.04-A—INITIAL PERFORMANCE AND RECOVERY (IPR) AND ONGOING PERFORMANCE AND RECOVERY (OPR) FOR VIABLE *ASCARIS* OVA

15. Action Requirement

15.1 Each time a modification is made to this method, the laboratory is required to perform the IPR test and the matrix spike/matrix spike duplicate (MS/MSD) test to demonstrate that the modification produces results equivalent or superior to results produced by this method.

16. Initial precision and recovery (IPR)

16.1 To establish the ability to demonstrate control over the analytical system and to generate acceptable precision and recovery, the laboratory shall perform the following operations:

16.1.1 Using enumerated spiking suspension aliquots containing 100 to 200 viable ova, the laboratory must elute, separate, and examine four 300-g compost samples. If more than one process will be used for separation of samples, a separate set of IPR samples must be prepared for each process.

NOTE 1I—IPR tests must be accompanied by analysis of a method blank, presented in step 18.

16.1.2 Using results of the four analyses, compute the average percent recovery (R) and the standard deviation of the recovery (s_r) for *Ascaris* Ova.

Note 2I—Acceptance criteria have not yet been determined for this method. We are seeking data generated through interlaboratory validation of this method using compost samples spiked with fecally derived *Ascaris* ova.

17. Matrix Spike (MS) and Matrix Spike Duplicate (MSD)

17.1 *Matrix spike, MS*—The laboratory shall spike and analyze a separate field sample aliquot to determine the effect of the matrix on the method's viable ova recovery. The MS shall be analyzed according to the frequency in Step 10 (Procedure for Method A).

17.1.1 Analyze an unspiked field sample according to the Procedures for Method A, Step 10. Using the spiking procedure and an appropriate volume of enumerated viable ova spiking suspension, spike a second field sample aliquot to produce five times the number of viable ova detected in the unspiked sample or the number used in the IPR or OPR tests (Steps 16 and 19), whichever is greater.

17.1.2 Compute the percent recovery (R) of *Ascaris* ova using the following equation:

$$R = 100 \times (S - U) \div T \qquad \textit{Equation 17.1.2}$$

where:

R = percent recovery,
S = number of viable ova detected in the spiked sample,
U = number of viable ova detected in the unspiked sample, and
T = true value of the viable ova spiked.

17.1.3 Calculate the percent recovery (R) for *Ascaris* Ova using Equation 17.3.1. Calculate the mean of the MS- recoveries and MSD-recoveries.

$$X = [MS + MSD] \div 2 \qquad \textit{Equation 17.1.3}$$

17.1.4 Calculate the relative percent difference (RPD) of the recoveries using the following equation:

$$RPD = 100 \times |RMS - RMSD| \div X \qquad \textit{Equation 17.1.4}$$

where:

RPD = relative percent difference,
RMS = number of viable ova detected in the MS,
RMSD = number of viable ova detected in the MSD, and
X = mean of the recoveries for the MS and MSD.

18. Method Blank

18.1 *Negative Control Sample*—Reagent water blanks are analyzed to demonstrate freedom from contamination. Analyze the blank immediately prior to analysis of the IPR test (step 16) and OPR test (step 19) and prior to analysis of samples for the week to demonstrate freedom from contamination.

18.1.1 Filter, elute, concentrate, separate (purify), stain, and examine at least one reagent water blank per week according to step 10, Procedures for Method A. If more than 20 samples are analyzed in a week, process and analyze one reagent water blank for every 20 samples.

18.1.2 If viable *Ascaris* ova or any potentially interfering organism or material is found in the blank, analysis of additional samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. Any sample in a batch associated with a contaminated blank that shows the presence of one or more viable ova is assumed to be contaminated and must be recollected. Any method blank in which viable ova are not detected is assumed to be uncontaminated and may be reported.

19. Ongoing Precision and Recovery, (OPR)

19.1 *Positive Control Sample or Laboratory Control Sample*—Using the spiking procedure and enumerated spiking suspension containing 100 to 200 viable ova (Method 07.04-A), filter, elute, concentrate, separate (purify), stain, and examine at least one spiked compost sample per week to verify all performance criteria. The laboratory must analyze one OPR sample

for every 20 samples if more than 20 samples are analyzed in a week. Adjustment and/or recalibration of the analytical system shall be performed until all performance criteria are met. Only after all performance criteria are met may samples be analyzed.

19.2 Examine the slide from the OPR prior to analysis of samples from the same batch.

19.2.1 More than 50% of the viable ova must appear undamaged and morphologically intact; otherwise, the analytical process is damaging the viable ova. Determine the step or reagent that is causing damage to the viable ova. Correct the problem and repeat the OPR test.

19.2.2 Identify and. enumerate each organism. Each organism must meet the identification criteria in step 10.2.3.

19.3 Compute the percent recovery (R) of the total number of viable ova using the following equation:

$$R = 100 \times N \div T \qquad \textit{Equation 19.3}$$

where:

N = number of viable ova detected, and

T = number of viable ova spiked

19.3.1 *Microscope System*—To determine if the failure of the OPR test (Step 17) is due to changes in the microscope, examine a slide containing a known number of freshly prepared viable ova.

19.3.2 *Elution/concentration System*—If the failure of the OPR test (Step 17) is attributable to the elution/concentration system, these systems may not be in control. Check filtration/elution/concentration system performance using spiked reagent water and analyzing the sample without separation (purification).

19.4 The laboratory should add results that pass the specifications in Step 17 to initial and previous ongoing data and update the QC chart to form a graphic representation of continued laboratory performance. The laboratory should develop a statement of laboratory accuracy (reagent water, raw surface water) by calculating the average percent recovery (R) and the standard deviation of percent recovery (s,). Express the accuracy as a recovery interval from $R-2 \cdot s_r$ to $R+2 \cdot s_r$. For example, if R = 95% and s, =25%, the accuracy is 45% to 145%.

20. Proficiency Testing

20.1 The laboratory should periodically analyze external QC samples, such as a performance evaluation or standard reference materials, when available. The laboratory also should periodically participate in inter-laboratory comparison studies using the method.

Test Method: Recovery and Assay of Total Culturable Viruses. Referenced Only						**Units:** PFU $(4 \cdot g)^{-1}$		
Test Method Applications								
Process Management							**Product Attributes**	
Step 1: Feedstock Recovery	*Step 2:* Feedstock Preparation	*Step 3:* Composting	*Step 4:* Odor Treatment	*Step 5:* Compost Curing	*Step 6:* Compost Screening and Refining	*Step 7:* Compost Storing and Packaging	*Safety Standards*	*Market Attributes*
				07.05		07.05	07.05	

07.05 RECOVERY AND ASSAY OF TOTAL CULTURABLE VIRUSES

DISCLAIMERS

(1) The methodologies described in TMECC do not purport to address all safety concerns associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use.

(1) All methods and sampling protocols provided in TMECC are subject to revision and update to correct any errors or omissions, and to accommodate new widely accepted advances in techniques and methods. Please report omissions and errors to the U.S. Composting Council Research and Education Foundation. An on-line submission form and instructions are provided on the TMECC web site, http://tmecc.org/.

(2) Process alternatives, trade names, or commercial products as mentioned in TMECC are only examples and are not endorsed or recommended by the U.S. Department of Agriculture or the U.S. Composting Council Research and Education Foundation. Alternatives may exist or may be developed.

1. Significance

1.1 This test covers the recovery and assay of virus from water, biosolids, soil and compost. The protocols are used to demonstrate that the material meets the requirement that human enteric viruses, i.e., viruses that are transmitted via the fecal-oral route, are less than one plaque-forming unit (PFU) per four grams of total solids.

1.2 Compost to be monitored may contain pathogenic human enteric viruses. Laboratories performing virus analyses are responsible for establishing an adequate safety plan and must decontaminate and dispose of wastes according to their safety plan and all applicable regulations. Aseptic techniques and sterile materials and apparatus must be used throughout the method.

2. Selection of Method

2.1 *Sample Analysis:*

2.1.1 EPA625R92013. Control of Pathogens and Vector Attraction in Sewage Sludge, Appendix H. Method for the Recovery and Assay of Total Culturable Viruses from Sludge. ed. Shay Fout – Biohazard Assessment Research Branch, National Exposure Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, USA 45268. This publication is available on-line at http://tmecc.org/documents/625r92013.pdf, modified from EPA600/4-84/013(R7), September 1989 Revision (section 3).

EPA600484013. USEPA Manual of Methods for Virology. The electronic text form of the manual consists of 12 chapters and are available on-line at www.epa.gov/nerlcwww/about.htm.