Designation: G160 – 12

Standard Practice for Evaluating Microbial Susceptibility of Nonmetallic Materials By Laboratory Soil Burial[1]

This standard is issued under the fixed designation G160; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice is limited to the method of conducting an evaluation of a nonmetallic material's microbiological susceptibility when in contact with the natural environment of the soil under use conditions. This practice is intended for use on solid material test specimens that are no larger than approximately 2 cm (0.79 in.) thick and 100 cm^2 (15.5 $in.^2$) or on film forming materials such as coatings which may be tested in the form of films at least 50 by 50 mm (2 by 2 in.) in size. This practice may be applied to articles that do not spend the majority of their service life in soil.

1.2 A wide variety of properties may be affected by microbial attack depending on material or item characteristics. Standard methods (where available) should be used for each different property to be evaluated. This practice does not attempt to enumerate all of the possible properties of interest nor specify the most appropriate test for those properties. Test methods must, however, be appropriate to the material being tested.

1.3 Materials intended for use in soil burial applications are often subjected to periods of exposure to solar radiation and other elements of weather for some time before they are buried. Because these exposures may alter the ability of a material to resist the effects of soil-borne microorganisms, it is recommended that this practice be combined with appropriate environmental exposures (for example, solar simulating weathering devices, the hydrolytic effects of extended aqueous contact, or extraneous nutrients) or fabrication into articles (for example, adhesive bonding of seams) which may promote microbiological susceptibility during the service life of the material.

1.4 The values stated in SI units are to be regarded as standard. The values given in parentheses are provided for information purposes only.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

G154 Practice for Operating Fluorescent Ultraviolet (UV) Lamp Apparatus for Exposure of Nonmetallic Materials

G155 Practice for Operating Xenon Arc Light Apparatus for Exposure of Non-Metallic Materials

3. Significance and Use

3.1 These results may be used to compare the susceptibility of materials when exposed to this test procedure.

3.2 Microbiological susceptibility may be reflected by a number of changes including staining, weight loss, or reduction in tensile or flexural strength.

3.3 This practice may be considered an inoculation with a mixed culture of fungi and bacteria.

4. Soil

4.1 *Composition*— Soil shall be composed of equal parts of fertile topsoil (soil with a high clay content should not be used), well-rotted and shredded horse manure, and coarse sand (10 to 40 mesh).

4.2 *Mixing*—The soil composition of 4.1 should be prepared by simple mixing and sifting through ¼-in. mesh screen.

4.3 *Aging*—The mixture is aged for three months and resifted twice at four-week intervals during the three months. After three months, a viability control of untreated cotton cloth, 400 to 475 g/m^2 (12 to 14 oz/yd^2), buried in the soil shall have a tensile strength loss of at least 50 % after five days.

NOTE 1—The soil mixture may be used for sequential tests as long as the cotton cloth control degrades within the specified time period.

4.4 *pH*—The soil shall have a pH between 6.5 to 7.5, checked periodically, and maintained by the addition of ground

[1] This practice is under the jurisdiction of Committee G03 on Weathering and Durability and is the direct responsibility of Subcommittee G03.04 on Biological Deterioration.

Current edition approved Nov. 1, 2012. Published January 2013. Originally approved in 1998. Last previous edition approved in 2009 as G160 – 03(2009). DOI: 10.1520/G0160-12.

[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

limestone to raise the pH or flowers of sulfur to lower the pH. The soil pH may be taken by dispersing 1 weight part soil in 20 parts of water, shaking or stirring, then allowing the mix to settle for 1 h. The pH is measured with indicator paper, electrodes, or by titration.

4.5 *Moisture*—The soil shall be maintained at between 20 and 30 % moisture, based on the dry weight of the soil. (The percent moisture is calculated by weighing approximately 50 mL of a representative portion and taking the portion to constant weight by placing the soil in an oven at a temperature of 101 to 106°C.) Water lost during use as a result of evaporation shall be replaced without deforming the soil bed. If the surrounding atmosphere is maintained at 85 to 95 % relative humidity, this loss is negligible, however, the moisture level should be periodically measured.

5. Apparatus

5.1 *Soil Container*— The container shall be any material of suitable mechanical strength and chemical/microbial resistance and, if porous, shall be lined with impermeable material. It shall be of any size that is convenient to handle and having a depth of at least 12.7 cm (5 in.).

5.2 *External Environment*—An apparatus capable of maintaining a temperature of 30 ± 2°C (86 ± 3.6°F) and a relative humidity to 85 to 95 %, into which the assembled container is inserted, is necessary. An incubator or controlled tropical chamber is adequate.

6. Test Specimens

6.1 Completely fabricated parts or sections cut from fabricated parts may be used as test specimens. The simplest specimen may be a 50- by 50-mm (2- by 2-in.) piece, a 50-mm (2-in.) diameter piece, or a piece (rod or tubing) at least 76 mm (3 in.) long cut from the material to be tested.

6.2 Film-forming materials such as coatings may be tested in the form of films at least 50 by 50 mm (2 by 2 in.) in size. Such films may be prepared by casting on glass and stripping after cure or by impregnating (completely covering) filter paper or ignited glass fabric.

6.3 For visual evaluation, a minimum of three test specimens shall be used.

6.4 In devising a test program intended to reveal quantitative changes occurring during and after exposure, an adequate number of specimens should be evaluated to establish a valid value for the original property. For example, if five replicate test specimens are required to establish a tensile strength of a film material, at least that number of test specimens shall be removed and tested for each exposure period. It is to be expected that values of physical or mechanical properties at various stages of fungal attack will be variable. The ASTM *Manual 7*[3] may be used as a guide.

[3] *Manual on Presentation of Data and Control Chart Analysis* , 6th ed., *Manual 7*, American Society for Testing and Materials, 1990.

7. Solar-Simulating Weathering Devices

7.1 For those soil burial applications in which the materials are expected to be exposed to environmental conditions, including solar radiation, it is recommended that they be exposed in a laboratory accelerated weathering device prior to soil burial. The type and duration of exposure shall be agreed upon by interested parties. The duration shall be of sufficient length, known by previous experience to have reduced the resistance of some materials to the effects of soil-borne microorganisms. If used, the type and duration of exposure shall be completely described in the Test Report.

7.2 The two types of weathering devices described in the Practices listed below have been used historically prior to evaluation of the microbial susceptibility of nonmetallic materials by laboratory soil burial. Because of differences in the spectral power distributions of the exposure sources as well as the other conditions in the two types of laboratory weathering tests, they may produce different test results or require different exposure durations for equivalent effects. The two types of exposure cannot be used interchangeably without supporting data that demonstrates equivalency of the exposures for the materials tested.

7.2.1 Xenon arc with daylight filters exposures conducted according to Practice G155 Cycle 7A.

7.2.2 Fluorescent UVA- 340 exposures conducted according to Practice G154 Cycle 1.

8. Viability

8.1 The viability must be recommended as in 4.3 concurrent with the test specimen and exposed consistent with the test specimen duration and number of test specimens.

9. Replicates

9.1 A minimum of four replicates are recommended.

10. Duration

10.1 The exposure period for soil burial, unless otherwise specified, shall be for a minimum of 60 days.

NOTE 2—The test specimens cannot be removed from the soil bed, once they have been buried, until the exposure period has been completed. Disturbing the soil bed in such a manner as removing the test specimens may affect the growth of soil microbes and thus cause inconsistent results. A separate set of specimens must be used for each exposure interval (for example, a set of replicates for 30, 60, and 90 days as needed).

11. Calculation and Interpretation of Results

11.1 *Visual*—At the end of the exposure period, the specimens shall be removed from the soil bed and conditioned according to methods appropriate to the material being tested. For visual evaluation, the material is rinsed under a stream of tap water while gently rubbing between fingers to remove soil and air-drying at 20°C (68°F) for minimum amount of time. Alternatively, the material may be gently vacuumed or very gently air-brushed.

11.2 Microbial staining shall be evaluated as follows:

Observed Growth or Stain	Rating
None	0
Trace (less than 10 % coverage)	1
Light (10 to 30 % coverage)	2
Moderate (30 to 60 % coverage)	3
Heavy (60 % to complete coverage)	4

11.3 *Property Changes*— Physical and mechanical changes such as tensile strength, flexibility, weight loss, or other tests, may be performed as described in appropriate ASTM or other test methods. Tests shall be conducted on unexposed and exposed specimens for the purpose of comparison in determining the extent of microbial degradation of the test material.

11.4 Calculate the change in property for each replicate specimen using one of the following equations:

$$C_{e,\,i} = X_{e,\,i} - \bar{X}_o \quad (1)$$

$$C_{e,\,i} = X_{e,\,i} - \bar{X}_f \quad (2)$$

where:

$C_{e,\,i}$ = change in property of each exposed specimen
$X_{e,\,i}$ = measured property of each exposed specimen,
$\bar{X}_o$ = mean of property from initial measurements on unexposed specimens, and
$\bar{X}_f$ = mean of property from measurements on file specimens.

11.5 Use the following equation to determine the mean change in property:

$$\bar{C} = \frac{\sum_{i=1}^{n} C_{e,\,i}}{n} \quad (3)$$

where:

n = number or exposed specimens.

11.6 Use the following equation to determine the standard deviation of the change in property:

$$S_c = \sqrt{\frac{\sum \left(C_i - \bar{C}\right)^2}{n-1}} \quad (4)$$

12. Report

12.1 Report the following information: age of bed, specimen size, number of replicates, performance of viability control, visual staining in accordance with 11.1 and 11.2 or property change in accordance with 11.3, duration of burial, and any specific measurements requested.

12.2 If used, report the exposure test and duration and a complete description if the exposure test is different from those recommended in Section 7.

12.3 Satisfactory or unsatisfactory performance of a material is dependent on the applicable standard for that material or methods agreed upon between the investigators.

13. Keywords

13.1 biodegradeable; biological deterioration; defacement; disfigurement; fungal resistance; fungi; laboratory soil culture; microbial susceptibility; microbiological deterioration; mildew; mixed microbial innoculum; mold growth; soil environment