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Soil quality — Test for estimating organic matter decomposition in contaminated soil

Qualité du sol — Essai d'estimation de la décomposition de la matière organique dans un sol contaminé

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Determining if the soil microbial community is healthy is a complex task and is influenced by the community's resistance and resilience to disturbances[1]. In its simplest terms, a healthy soil is one that functions in organic matter decomposition (OMD) and nutrient cycling. In fact, a suite of standardized test methods are needed to better understand the ecology of the soil microbial community and its role in soil function and structure[2]. However, one key soil microbiological function is organic matter decomposition. Unfortunately, there is a lack of standardized procedures for quantitatively measuring this important process. As such, the ability of soil microorganisms to decompose lignin cellulosic material provides evidence that the microbial population is active in OMD and carbon cycling. A standard field method currently available for assessing soil OMD inhibition from environmental contaminants involves using litter bags placed in experimental plots[3]; however, there is no standard method available for a laboratory-based assessment of organic matter decomposition. A laboratory-based method has been developed using the same principles as the litterbag method. In place of indigenous organic matter (i.e. tree leaves, crop material, etc.), the laboratory-based method uses readily accessible filter paper as a standard organic material for organic matter decomposition tests[4]. The laboratory-based method has been used and described in several research studies as part of a greater soil microbial health (SMH) assessment suite of tests[5],[6]. The studies evaluated the impact of contaminants in soil from brownfield sites or from testing of chemical-spiked control soil for risk assessment research.

This document outlines a procedure for determining the effects of contaminated soils on the decomposition of organic matter (lignin cellulosic filter paper) following a standardized methodology.

Soil quality — Test for estimating organic matter decomposition in contaminated soil

WARNING — Contaminated soils may contain unknown mixtures of toxic, radiotoxic, genotoxic, mutagenic, or otherwise harmful chemicals or infectious microorganisms. Occupational health risks may arise from dust or evaporated chemicals during handling and incubation. Precautions should be taken to avoid skin contact.

IMPORTANT — The electronic file of this document contains colours which are considered to be useful for the correct understanding of the document. Users should therefore consider printing this document using a colour printer.

1 Scope

This document specifies a test procedure for the evaluation of the habitat function of soils by determining effects of soil contaminants and substances on organic matter decomposition. This test is applicable to natural soils and soil materials of unknown quality (e.g. contaminated sites, amended soils, soils after remediation, agricultural or other sites under concern). This document also specifies how to use this method for testing substances under temperate conditions.

This document is not applicable to substances for which the air/soil partition coefficient is greater than 1. It is not applicable to substances with vapour pressure exceeding 300 Pa at 25 °C.

NOTE The stability of the test substance cannot be ensured over the test period. No provision is made in the test method for monitoring the persistence of the substance under test.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10390, *Soil, treated biowaste and sludge – Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11265, *Soil quality — Determination of the specific electrical conductivity*

ISO 11277, *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
contaminant
substance or agent present in the soil as a result of human activity

3.2
EC*x*
effective concentration
concentration (mass fraction) of a test sample or test substance that causes *x* % of an effect on a given endpoint within a given exposure period

Note 1 to entry: The EC*x* is expressed as a percentage of soil to be tested (dry mass) per soil mixture (dry mass). When substances are tested, the EC*x* is expressed as mass of the test substance per dry mass of soil in milligrams per kilogram. This can only be determined for chemically-spiked soil.

3.3
limit test
single concentration treatment consisting of at least five replicates for:

a) the *test soil* (3.8) or the highest concentration of test substance mixed into the *control soil* (3.7), and

b) the control soil

3.4
LOEC
lowest observed effect concentration
lowest test substance concentration that has a statistically significant effect (probability $p < 0,05$)

Note 1 to entry: In this test, the LOEC is expressed as a mass of test substance per dry mass of the soil to be tested. All test concentrations above the LOEC should usually show an effect that is statistically different from the control. This can only be determined for chemically-spiked soil.

3.5
NOEC
no observed effect concentration
highest test substance concentration immediately below the *LOEC* (3.4) at which no effect is observed

Note 1 to entry: In this test, the concentration corresponding to the NOEC, has no statistically significant effect (probability $p > 0,05$) within a given exposure period when compared with the control. This can only be determined for chemically-spiked soil.

3.6
reference soil
uncontaminated soil with comparable pedological properties (nutrient concentrations, pH, organic carbon content and texture) to the *test soil* (3.8) being studied

3.7
control soil
reference soil (3.6) used as a control against chemically-spiked *test soil* (3.8), which fulfils the validity criteria

Note 1 to entry: it is advisable that a control soil be proven to be suitable for use by demonstrating the ability of this soil to meet the standard's test validity criteria prior to definitive testing.

Note 2 to entry: Control soil cannot be artificial soil (AS) as it is known that this type of soil does not meet the validity criteria of the test.

3.8
test soil
sample of field-collected soil or chemically-spiked soil to be evaluated for toxicity to the soil microbial community

4 Principle

The ability of soil microorganisms to degrade cellulose filter paper (i.e. organic matter) in test soil (i.e. contaminated soil) is compared to the same cellulose filter paper in control or reference soil over an incubation period. In this method, the effects of individual substances can be assessed using standard natural soil used in chemical-spiking testing. For contaminated soils, the effects are determined in the test soil and in a control soil. This laboratory method uses sterilized filter paper disks as a source of organic matter. Organic matter decomposition is estimated based on the mass loss of filter paper disks placed between two layers of the test soil. If the contaminant in any way impairs the soil microorganism's ability to degrade cellulose filter paper through carbon cycle enzymes, a degradation rate difference is observed between the test soil and control treatments. Filter paper was chosen as the organic matter test material as it is universally available, is a more standardized media, allows for better inter-laboratory reproducibility, can be sterilized (e.g. autoclaved) and can be easily distinguished from native organic matter in the soil[4],[9].

The test involves pre-weighing filter paper disks, adding 10 g of soil on a dry mass basis to a 50 ml sterile centrifuge tube, placing the filter paper on top of the soil, adding an additional 10 g of soil (dry mass) on top of the filter paper, loosely capping the tube and incubating it at a constant temperature (e.g. 20 °C ± 2 °C).

For control versus contaminated soil design, the test is performed with a minimum of five replicates for each treatment with a minimum of five sampling time points. The incubation period depends on the degradation rate of filter paper, so the test length is tied to the extent of filter paper mass loss in the control treatment. A minimum mass loss in the control of 30 % is the earliest point for consideration of test completion but between 40 % and 70 % degradation is the ideal range. Beyond this range, it is more likely that the decomposed filter paper be difficult to clean and recover for mass loss measurements. The time interval between sampling time points is dependent on the degree of microbial activity in the control or reference soil.

In the case where soil is spiked with a contaminant, initial range-finding testing is advisable using a broad concentration range of the contaminant. For the definitive chemical dilution series, a minimum of 5 test concentrations is recommended. The duration of the test is influenced by the control and the time needed to observe a distinct concentration response. From experience, the test should not exceed 140 days.

5 Reagents and material

5.1 Reagents

5.1.1 Sterile deionized (or distilled) water.

5.2 Materials

5.2.1 50 ml plastic centrifuge tubes (sterile).

5.2.2 Cellulose filter paper; particle retention, ≥ 11 µm to 25 µm; thickness, ~180 µm; ash content, ~0,06 %; (sterile).

5.2.3 Hole punch (25 mm diameter).

5.2.4 Filter paper forceps (sterile).

5.2.5 Petri dishes (glass or plastic).

5.2.6 Small paint brushes (soft bristles).

5.2.7 Large plastic weigh-boats or alternate vessel.

5.2.8 Small aluminium weigh-boats or alternate vessel.

6 Soil

6.1 Field-collected soil

Field-collected soils, contaminated and control (i.e. reference), can be obtained from industrial, agricultural, boreal forest or other contaminated sites of concern. In the case of undisturbed soil, the organic horizon is used. The soil is shipped to the laboratory and stored at 4 °C.

All field-collected soils shall be passed through a 2 mm sieve. If required, soils may be slightly air-dried just to enable sieving; however, where possible, air-drying should be avoided. After sieving, the soil is homogenized and then stored again at 4 °C. Soil should be stored using containers that minimize losses of soil contaminants by volatilization and sorption to the container walls. Variable storage periods are possible for this test, so long as microbial activity is evident in the control or reference soil by meeting the validity criteria of the test. Soil pH, conductivity, moisture content and water holding capacity (WHC) are determined as per the methods below.

For interpretation of test results, the following characteristics shall be determined for each soil sampled from a field site:

a) pH in accordance with ISO 10390;

b) texture (sand, loam, silt) in accordance with ISO 11277;

c) water content in accordance with ISO 11465;

d) organic carbon in accordance with ISO 10694;

e) specific electrical conductivity in accordance with ISO 11265;

f) water holding capacity in accordance with Annex A.

6.2 Control soil

The control soil can be the reference soil in the context of contaminated soil assessment. The reference soils from an uncontaminated area near a contaminated soil site should be handled and shipped, and characterized in a manner similar to the contaminated test soils. In the case of a chemically-spiked soil study, a known control soil is used.

7 Apparatus

Use laboratory equipment and the following.

7.1 Top-loading balance.

7.2 Apparatus to determine the dry mass of the substrate, in accordance with ISO 11465 (drying oven, desiccator, analytical balance).

7.3 Digital camera (optional).

7.4 Desiccant chamber.

7.5 pH meter.

7.6 Analytical balance, capable of weighing with an accuracy of ±0,000 1 g.

7.7 Drying oven, set to (105 ± 5) °C.

7.8 Test environment.

7.8.1 Area to maintain a sterile environment, work bench with Bunsen burner or biological safety cabinet (optional).

7.8.2 Enclosure, capable of constant temperature control.

8 Procedure

8.1 Experimental design

8.1.1 General

A sample of field collected contaminated soil at a single concentration or a chemically-spiked soil at multiple concentrations are compared to an appropriate reference or control soil. Various test designs are described in 8.1.2 and 8.1.3. However, regardless of the test design chosen, each test concentration and associated control soil is replicated five times to allow for the time-spaced sampling during the duration of this testing standard. A filter paper disk (i.e. organic matter) is added to the test soil and the mass loss of filter paper determined over an incubation period.

8.1.2 Chemically-spiked soil test design

8.1.2.1 Range-finding

A preliminary test is recommended to find the range of concentrations that brackets the effect level of a new test substance (e.g. 0 mg/kg, 1 mg/kg, 10 mg/kg, 100 mg/kg and 1 000 mg/kg). A range-finding test shall be performed using the same batch of soil as the planned definitive test. The testing can be conducted with reduced replication (e.g. 2 or 3 replicates), relative to the definitive test. The duration of the range-finding test is the same as for the definitive test. The concentration range in the definitive test should preferably be chosen so that it includes concentrations that span a wide range, including a low concentration that evokes no adverse effects (similar to the negative control treatment) and a high concentration that results in severe effects. Ideally, the concentrations chosen also brackets the mid-range effects to better estimate the EC50 effect concentration.

When no effects are observed in a range-finding test, either with a 100 % contaminated soil sample or a spiked chemical test, the definitive test can be designed as a limit test (e.g. undiluted contaminated field soil) or a specific high chemical concentration (e.g. 1 000 milligrams of test substance per kilogram of test soil).

8.1.2.2 Chemically-spiked soil

In the case where chemical substances are spiked into the soil at different concentrations, the test can be designed for the following two scenarios:

a) For the EC*x* approach, a minimum of 5 concentrations plus the control treatment(s) shall be used, and more (i.e. ≥ 10 concentrations plus controls) are recommended to improve the likelihood of bracketing each end point sought according to ISO 10694. The dilution factor can be variable; smaller at lower concentrations, larger at high concentrations. A minimum of five replicates for each treatment plus the controls are recommended.

b) For the NOEC hypothesis approach, at least five concentrations in a geometric series shall be used according to ISO 10694. Five replicates for each treatment plus eight controls are recommended.