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Soil quality — Test for measuring the inhibition of reproduction in oribatid mites (*Oppia nitens*) exposed to contaminants in soil

*Qualité du sol — Essai de détermination de l'inhibition de la reproduction chez les acariens oribates (*Oppia nitens*) exposés aux contaminants dans le sol*

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Foreword

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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

Ecotoxicological test systems are applied to obtain information about the effects of contaminants in soil and are proposed to complement conventional chemical analysis (see ISO 15799[1] and ISO 17616[2]). ISO 15799 includes a list and short characterization of recommended and standardized test systems and ISO 17616 gives guidance on the choice and evaluation of the bioassays. Aquatic test systems with soil eluate are applied to obtain information about the fraction of contaminants potentially reaching the groundwater by the water path (retention function of soils), whereas terrestrial test systems are used to assess the habitat function of soils with regards to supporting soil biota and interactions within.

Mites (Acari) are a world-wide and diverse group of arthropods belonging to a sub-class of Arachnida with over 40 000 species recorded, divided into two super-orders (Acariformes and Parasitiformes). Due to their relative small size (a few µm to a few cm), they occupy specific ecological niches on plants as well as in soils[5].

In recent years, there has been an increase in the development of biological test methods for assessing contaminated soil, which has historically lagged behind that for other media (e.g., water and sediment). Ecotoxicology tests for soil are challenged, among other things, by the complexity of soil systems (e.g., lack of homogeneity) and the variety of exposure routes (e.g., via alimentary uptake, exposure to pore water or soil vapours, or direct contact with soil particles). A recently developed method (ISO 21285[3]) assesses the effects of contaminated soil on the reproduction of the predatory mite (*Hypoaspis aculeifer*), mainly through alimentary uptake. Oribatid mites represent a different but essential ecological niche than *H. aculeifer* within soil, contributing to carbon mineralization and soil formation, as well as nitrogen and phosphorous release through grazing activities. Oribatid mites are among the most diverse and abundant micro-arthropod species within soil, however, their slower metabolism and development, coupled with low fecundity, long life span, and limited dispersal capacity increase the potential for susceptibility and sensitivity to short- and long-term disturbances[6]. The use of oribatid mites in the context of soil ecotoxicology testing has been thoroughly reviewed[7][8][9][10][11]. Recent research using *Oppia nitens* for soil testing has demonstrated applicability and relative sensitivity of the species for the assessment of contaminated soils from both agronomic regions, and those from the boreal and taiga ecozones[6][12][13][14][15]. Research has also demonstrated its sensitivity to metals[16][17][18][19], pesticides[20][21], and organic compounds[16][17][22]. *Oppia nitens* is an oribatid mite, inhabiting the upper organic layer of soil, and is a member of the largest oribatid family (Oppiidae) with approximately 1 000 species in 129 genera widely distributed throughout Holarctic and Antarctic regions[23]. They are sexually reproducing, polyphagous fungivores that can be easily reared in the laboratory in soil or on plaster of Paris, and on a diet of Baker's yeast[10].

This method outlines procedures for conducting 28-day tests for determining the effects of contaminated soils on the survival and reproduction of the oribatid mite, *Oppia nitens*. Optionally, the method can be used for testing chemicals added to standard soils (e.g., artificial soil) for their lethal and sublethal hazard potential to oribatid mites. The performance of this method has been assessed in an international inter-laboratory investigation[15], as summarized in Annex E. Mites represent communities that cannot be omitted from environmental hazard assessment. This species is considered to be representative of non-predatory soil mites.

Soil quality — Test for measuring the inhibition of reproduction in oribatid mites (*Oppia nitens*) exposed to contaminants in soil

WARNING — Contaminated soils may contain unknown mixtures of toxic, 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.

1 Scope

This document specifies one of the methods for evaluating the habitat function of soils and determining effects of soil contaminants and individual chemical substances on the reproduction of the oribatid mite *Oppia nitens* by dermal and alimentary uptake. This chronic (28-day) test is applicable to soils and soil materials of unknown quality (e.g., contaminated sites, amended soils, soils after remediation, agricultural or other sites under concern and waste materials). This method is not intended to replace the earthworm or Collembola tests since it represents another taxonomic group (= mites; i.e., arachnids), nor the predatory mite test since this species represents a different trophic level and ecological niche.

Effects of substances are assessed using standard soil, preferably a defined artificial soil substrate. For contaminated soils, the effects are determined in the test soil and in a control soil. According to the objective of the study, the control and dilution substrate (dilution series of contaminated soil) should be either an uncontaminated soil with similar properties to the soil sample to be tested (reference soil) or a standard soil (e.g., artificial soil).

Information is provided on 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, or to substances with vapour pressure exceeding 300 Pa at 25 °C.

NOTE The stability of the test substance cannot be assured 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 quality — Determination of pH*

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

ISO 11260, *Soil quality — Determination of effective cation exchange capacity and base saturation level using barium chloride solution*

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*

ISO 18400-206, *Soil quality — Sampling — Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

3 Terms and definitions

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

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

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

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

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

3.2
effect concentration
EC*x*
concentration (mass fraction) of a test sample or test substance that causes *x* % of an effect on a given end-point within a given exposure period when compared with a control

EXAMPLE An EC50 is a concentration estimated to cause an effect on a test end-point in 50 % of an exposed population over a defined 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.

3.3
effect rate
ER*x*
rate of a soil to be tested that causes an *x* % of an effect on a given end-point within a given exposure period when compared with a control

3.4
lethal concentration
LC*x*
concentration (mass fraction) of a test sample or test substance that causes *x* % mortality within a given exposure period when compared with a control

EXAMPLE An LC50 is a concentration estimated to cause mortality in 50 % of an exposed population over a defined exposure period.

Note 1 to entry: The LC*x* is expressed as a percentage of soil to be tested (dry mass) per soil mixture (dry mass). When substances are tested, the LC*x* is expressed as mass of the test substance per dry mass of soil in milligrams per kilogram.

3.5
limit test
single concentration test consisting of at least five replicates each, the *test soil* (3.14) without any dilution or the highest concentration of test substance mixed into the control soil and the control

3.6
lowest observed effect concentration
LOEC
lowest tested concentration (mass fraction) of a test sample or test substance that has a statistically significant effect (probability $p < 0,05$)

Note 1 to entry: In this test, the LOEC is expressed as a percentage of soil to be tested (dry mass) per soil mixture (dry mass) or 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.

3.7
lowest observed effect rate
LOER
lowest rate of a soil to be tested in a control soil at which a statistically significant effect is observed

3.8
no observed effect concentration
NOEC
highest tested concentration (mass fraction) of a test sample or test substance immediately below the LOEC 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.

3.9
no observed effect rate
NOER
lowest rate of a soil to be tested immediately below the LOER which, when compared to the control, has no statistically significant effect (probability $p < 0,05$) within a given exposure period

3.10
reproduction
mean number of offspring per test vessel after a 28-day incubation under the specified test conditions

3.11
reference soil
uncontaminated soil with comparable pedological properties (nutrient concentrations, pH, organic carbon content and texture) to the *test soil* (3.14)

3.12
standard soil
field-collected soil or artificial soil whose main properties (pH, texture, organic matter content) are within a known range

EXAMPLE Euro soils, artificial soil, LUFA standard soil.

Note 1 to entry: The properties of standard soils can differ from those of the test soil.

3.13
control soil
reference or *standard soil* (3.12) used as a control and as a medium for preparing dilution series with test soils/samples or a reference substance, which fulfils the validity criteria

Note 1 to entry: In the case of natural soil, it is advisable to demonstrate its suitability for a test and for achieving the test validity criteria before using the soil in a definitive test.

3.14
test soil
sample of field-collected soil or chemical-spiked soil to be evaluated for toxicity to mites

3.15
test mixture
mixture of contaminated soil or the test substance (e.g. chemical, biosolid, waste) with *control soil* (3.13)

Note 1 to entry: Test mixtures are given in percent of contaminated soil based on soil dry mass.

3.16
test mixture ratio
ratio of test soil to control soil in a *test mixture* (3.15)

Note 1 to entry: Different ratios may be applied in a dilution series to establish a dose-response relationship.

4 Principle

The effects on reproduction of adult, laboratory-cultured mites, *Oppia nitens,* exposed to the test soil are compared to those observed for organisms in control soil. If appropriate, effects based on exposure to a test mixture of contaminated soil and control soil, or a range of concentrations of a test substance mixed into control soil, are determined. Test mixtures are prepared at the start of the test and are not renewed during the test period.

The test is started with 15 adult mites from age-synchronized cultures (aged 8 to 10 d after ecdysis (i.e., moult) to adult form) per test vessel. The test is performed in 30 mL glass vessels with a wet-weight equivalent to a volume of ~20 mL of soil, and a minimum of five replicates are prepared for each treatment. The test runs for 28 d at (20 ± 2) °C by which time offspring (F_1) have emerged from eggs laid by the adults and the number of offspring produced is determined. Survival of the adults is also determined at the end of the test. The results obtained from the tests are compared with a control or, where a serial dilution design is used, to determine the concentration resulting in *x* % reduction of juveniles produced compared to the control (EC*x*, 28 d), depending on the experimental design. An estimate of the test concentration resulting in *x* % mortality (LC*x*, 28 d) is an optional test endpoint. If a multi-concentration hypothesis test design is used, the reproductive output of the mites exposed to the test mixtures is compared to that of the controls in order to determine the concentration which causes no effects on mortality and reproduction (NOEC).

In cases where there is no prior knowledge of the dilution/concentration of the soil to be tested or the test substance likely to have an effect, then it is useful to conduct the test in two steps:

— a range-finding test is carried out to give an indication of the effect dilution/concentration, and the dilution/concentration resulting in no mortality. Dilutions/concentrations to be used in the definitive test can then be selected; and

— the definitive test to determine lethal and sub-lethal effects of (dilutions of) contaminated soil or the concentration of a substance which, when evenly mixed into the standard soil, results in: 1) *x* % inhibition of reproduction, EC*x* (e.g., EC10, EC20, or EC50), or 2) causes no significant effects on numbers of offspring hatched from eggs compared with the control for estimation of the NOEC and LOEC.

The use of a reference soil is an essential requirement to demonstrate the present status of the test population, and to avoid misinterpretation of results.

5 Reagents and material

5.1 Biological material

In this test, adult mites, *Oppia nitens* C.L. Koch 1836, aged 8 to 10 d (i.e., 8 to 10-d post-ecdysis to adult form), established from newly emerged adults collected over a 1- to 3-d period, are required to start the test. The mites shall be selected from an age-synchronised culture. A method for culturing *Oppia nitens* and for obtaining age-synchronised test organisms is provided in Annex A.

Adult *Oppia nitens* are obtained from laboratory cultures maintained under conditions of temperature, photoperiod, and food similar to those in the test. Species identification should be confirmed by qualified personnel experienced in identifying soil mites using the distinguishing taxonomic features, described in taxonomic keys[26], or using DNA-based taxonomic identification (i.e., barcoding) as outlined in ISO 21286[4]. All mites used in a test shall be derived from the same population and source. Sources of animals to be used to establish cultures include government or private laboratories that are culturing *Oppia nitens* for soil toxicity tests, or commercial biological suppliers[24].

5.2 Test mixture can consist of field-collected soil or control soil mixed with the test soil or spiked with the test substance.

5.2.1 Field-collected soil or waste

The sample(s) can be field-collected soil from an industrial, agricultural or other site of concern, or waste materials (e.g., dredged material, municipal sludge from a wastewater treatment works, plant-derived compost, or manure) under consideration for possible land disposal.

The field-collected soils used in this test shall be passed through a sieve of 4 to 10 mm square mesh to remove coarse fragments and thoroughly mixed. If necessary, soil may be air-dried without heating before sieving. Storage of the test soil should be as short as possible. The soil shall be stored in accordance with ISO 18400-206 using containers that minimize losses of soil contaminants by volatilization and sorption to the container walls. If soils or test mixtures have been stored, they should be mixed a second time immediately before use. Soil pH should not be corrected as it can influence bioavailability of soil contaminants.

NOTE A 4-mm mesh sieve is appropriate for use with any mineral-based soil with relatively low organic matter (e.g., agricultural soil), however for forest soils or wetland soils with higher organic matter content, sieves with larger mesh sizes (e.g., 6 to 8 mm for forest soils and 8 to 10 mm for wetland soils) could be required.

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) water holding capacity according to Annex B;

e) cationic exchange capacity in accordance with ISO 11260;

f) electrical conductivity in accordance with ISO 11265

g) organic carbon in accordance with ISO 10694;

h) percentage of material removed by the sieve.

NOTE It is important to measure the water holding capacity of all mixtures used in the test.

5.2.2 Control soil, either a) reference soil (3.11) or b) standard soil (3.12) that allows the presence of oribatid mites. Control soil and soil used for dilution shall not differ in one test (either a) or b)).

a) If reference soils from uncontaminated areas near a contaminated site are available, they should be treated and characterized like the test soils. If a toxic contamination or unusual soil properties cannot be ruled out, standard control soils should be preferred.

b) For testing the effects of substances mixed into soil, standard soils (e.g. artificial soil, LUFA standard soil) shall be used as test substrate. The properties of the field-collected standard soil shall be reported.