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Soil quality — Biological methods — Chronic toxicity in higher plants

Qualité du sol — Méthodes biologiques — Toxicité chronique sur les plantes supérieures

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 22030 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

This International Standard describes a procedure for evaluating the quality of soils of different origin carrying unknown contaminations. The method, slightly modified, can also be used to measure the toxicity of known chemicals incorporated into soil.

The evaluation of the inhibition and chronic toxicity is based on emergence, vegetative growth and reproductive capacity of at least two species of higher plants.

This International Standard is based on:

a) results of the research project “Development of a chronic bioassay using higher plants”, sponsored by the German Ministry for Education and Research (BMBF), Bonn [3], and

b) discussions within the joint project “Ecotoxicological Test Batteries” forming part of the BMBF Joint Research Group “Processes for the Bioremediation of Soil” [10].

Soil quality — Biological methods — Chronic toxicity in higher plants

WARNING — Contaminated soils can contain unknown mixtures of toxic, mutagenic or otherwise harmful chemicals or infectious microorganisms. Occupational health risks can arise from dust or evaporated chemicals during handling and incubation. Furthermore, test plants can absorb chemicals from the soil and safety measures should also be considered when handling these test plants.

1 Scope

This International Standard describes a method for determining the inhibition of the growth and reproductive capability of higher plants by soils under controlled conditions. Two species are recommended: a rapid-cycling variant of turnip rape (*Brassica rapa* CrGC syn. Rbr) and oat (*Avena sativa*). The duration of test should be sufficient to include chronic endpoints that demonstrate the reproductive capability of the test plants.

By using natural test soils, e.g. from contaminated sites or remediated soils, and by comparing the development of the test plants in these soils with reference or standard control soils, the test can be used to assess soil quality, especially the function of the soil as a habitat for plants.

Annex A describes modifications allowing use of the chronic plant assay for the testing of chemicals incorporated into soil. By preparing a dilution series of a test substance in standard control soils, the same endpoints can be measured to assess the chronic toxicity of chemicals. This method is not applicable to volatile substances, i.e. substances for which H (Henry's constant) or the air/water partition coefficient is greater than 1, or for which the vapour pressure exceeds 0,013 3 Pa at 25 °C.

2 Normative references

The following referenced documents are indispensable for the application 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 11268-1:1993, *Soil quality — Effects of pollutants on earthworms (Eisenia fetida) — Part 1: Determination of acute toxicity using artificial soil substrate*

ISO 11268-2:1998, *Soil quality — Effects of pollutants on earthworms (Eisenia fetida) — Part 2: Determination of effects on reproduction*

ISO 11269-2, *Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of chemicals on the emergence and growth of higher plants*

ISO 15176:2002, *Soil quality — Characterization of excavated soil and other soil materials intended for re-use*

ISO 15799, *Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials*

ASTM D1076:2002, *Standard Specification for Rubber-Concentrated, Ammonia Preserved, Creamed, and Centrifuged Natural Latex*

3 Terms and definitions

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

3.1
artificial soil
mixture of sand, kaolinite, peat and calcium carbonate

NOTE ISO 11268-1 describes such a soil for toxicity tests using earthworms. Pure quartz sand, mineral wool, vermiculite or other synthetic substrates should not be used.

3.2
biomass
total mass of shoots, flowers and seed pods

NOTE 1 Biomass is expressed as dry mass per plant or, if needed, as dry mass per pot.

NOTE 2 During the test period, some of the test plants can reach different growth stages and their water content can differ when the plants are harvested. Thus the dry mass better represents the biomass produced during the growth period.

3.3
concentration
mass of test substance per amount of soil

NOTE Concentration is expressed as a mass fraction, in milligrams per kilogram (mg/kg) of dry soil.

3.4
contaminant
substance or agent present in the soil as a result of human activity

[ISO 15176:2002]

3.5
control soil
uncontaminated substrate, used as a control and as medium for preparing dilution series with test soils or chemicals, that allows the growth of healthy plants

NOTE Either artificial or natural standard or reference soils can be used, if unhindered growth of the test plants in these soils can be expected. In any case, differences in nutrient levels between a test soil and a control soil can affect the dose-response pattern. For example, a control soil much richer in nutrients than a test soil can result in a false positive result (i.e. the test soil appears to have a “toxic” effect on the growth of the test plants). If a control soil is poorer in nutrients than a test soil, hormesis (see 3.9) can be expected at low soil-mixture ratios, or even an inverse dose response relationship, if nutrient supply becomes the main effect. This International Standard does not provide numerical values for the nutrients.

3.6
effect concentration
EC_x
concentration (mass fraction) of a test chemical or the percentage (mass fraction) of a test soil at which a given endpoint is inhibited by x % compared to the control

NOTE The effect concentration is expressed in milligrams per kilogram. When chemicals are tested, the EC_x is expressed as mass of the test substance per dry mass of soil; when soils are tested, the EC_x is expressed as a percentage of test soil dry mass per soil mixture dry mass.

3.7
emergence
development of a seedling contained within a seed, ending the latent period

NOTE It is expressed as the percentage of seedlings which emerge from test pots as compared with the control pots.

3.8
habitat function
ability of soils/soil materials to serve as a habitat for microorganisms, plants, soil-living animals and their interactions (biocenosis)

[ISO 15799]

3.9
hormesis
improvement of seedling emergence, growth or survival (or other response of the test plants) at low concentrations of chemicals or mixtures of soil that are toxic when applied at higher levels in comparison to the control [1]

3.10
lowest observed effect concentration
LOEC
lowest tested concentration (mass fraction) of a test substance in soil at which a statistically significant effect on a given endpoint ($p < 0{,}05$) compared with the control is observed

cf. **NOEC** (3.11)

NOTE Analogously, the term LOEC is used for the lowest tested mixture ratio of a test soil in a reference or a standard control soil at which a statistically significant effect is observed. The LOEC is expressed as mass of the test substance per mass of dry soil or, in the latter case, as percentage of test-soil dry mass per soil-mixture dry mass. All test concentrations above the LOEC have a harmful effect equal or greater than that observed at the LOEC. If this condition cannot be satisfied, an explanation should be given for how the LOEC and NOEC have been selected.

3.11
no observed effect concentration
NOEC
test substance concentration (mass fraction) or soil mixture ratio immediately below the LOEC, which when compared to the control has no statistically significant effect ($p < 0{,}05$)

cf. **LOEC** (3.10)

3.12
reference soil
uncontaminated site-specific soil (e.g. collected in the vicinity of a contaminated site) with properties (nutrient concentrations, pH, organic carbon content and texture) similar to the test soil

3.13
soil mixture ratio
ratio of the dry mass of test soil to the dry mass of reference/control soil

NOTE It is expressed as a percentage.

3.14
standard soil
field-collected soil or artificial soil whose main properties (e.g. pH, texture, organic matter content) are within a known range

EXAMPLES Euro soils, artificial soil.

NOTE The properties of standard soils may differ from those of the test soil.

4 Principle

This International Standard describes a plant test that includes both acute and chronic endpoints. The test measures emergence, early growth and reproduction of two terrestrial plant species (*Avena sativa* and a rapid-cycling variety of *Brassica rapa* are recommended). The test compares responses of plants in a test soil and/or a series of dilutions with a control soil. This test method may also be used for the testing of chemicals by applying various concentrations of the test substance to a standard control soil. Seeds of both plant species are planted in pots containing the soil/soil mixtures and in control pots containing a reference or standard soil. Pots are placed in a temperature- and light-controlled room or growth chamber. They are watered via wicks. After emergence of the plants, emergence rates are determined and plants are thinned out to a specified number. After two weeks, some of the plants are harvested to determine their biomass. After another period of three weeks to four weeks (rapid-cycling *Brassica rapa*) or five weeks to 6 weeks (*Avena sativa*), the remaining plants are harvested for measuring additional endpoints characterizing their reproductive potential. In all cases, the test duration should be sufficient to determine reproductive endpoints (e.g. number or biomass of flowers or seeds or fruit).

Typically, 10 seeds are sown in four replicate test pots each. Plants are thinned out to 8 per pot, and four plants each are harvested at day 14 and at the end of the test. If in any pot less than 8 plants have emerged, the number of plants harvested at day 14 shall be reduced such that four plants remain for the final harvest.

The relative inhibition in undiluted test soils is determined to assess the suitability of the soil for plants. In addition, based on a dilution series, NOEC, LOEC and EC_x values can be calculated from the dose response curves. The latter is required when chemicals are tested.

5 Materials

5.1 Test plants

One monocotyledonous and one dicotyledonous species are tested in parallel. Oat (*Avena sativa*) is recommended as the monocotyledonous and *Brassica rapa* as the dicotyledonous plant species.

To shorten the test period, a rapid-cycling variety of turnip rape (*Brassica rapa* CrGC syn. Rbr) is strongly recommended[1)]. Flowering starts after two weeks and seed production can be determined after approximately five weeks.

Other species may be selected, e.g. from the list given in ISO 11269-2 or plants with specific physiological characteristics such as C-4 plants (corn, sugar cane, millet), plants in symbiosis with nitrogen-fixing bacteria (e.g. *Fabaceae*) or plants with ecological or economic significance in certain regions of the world. These plants shall grow unhindered in control soil under the conditions specified. Only plants that tolerate the properties of the test soils and test conditions (including their chemical contamination) should be selected. For example, a species sensitive to low pH values should not be used for testing forest soils with low pH-values. Oat and rapid-cycling turnip rape grow in sandy as well as loamy soil with varying water content and a range of pH values from 5,0 to 7,5. Species that do not tolerate wet soils should not be used in combination with wick watering. Reasons for selecting species other than oat and turnip rape shall be justified in the test report.

5.2 Soil and soil storage

5.2.1 General

The description of methods for representative sampling of soils from contaminated sites is not within the Scope of this International Standard. A suitable sampling method is given in ISO 10381-6 [13].

1) Seeds and Wisconsin Fast Plants kits are suitable products supplied by the Carolina Biological Supply Company, Burlington, NC, USA. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

The chronic plant test can be used to assess the toxic potential of natural soils from a variety of contaminated sites. It can also be used to assess the quality of remediated soils. The selected soils should have pH values after sieving within a range that is not toxic to the test plants, e.g. between 5,0 and 7,5 for *Brassica rapa* and *Avena sativa*. Test soils shall be passed through a sieve of mesh 4 mm to 5 mm square to remove coarse fragments, and mixed thoroughly. If necessary, soil may be air-dried without heating before sieving. Storage of test soils should be as short as possible. Storage at approximately 4 °C using containers that minimize losses of soil contaminants by volatilization and sorption to the container walls is recommended.

5.2.2 Test soil

The water-holding capacity shall be measured for all soil mixtures used in the test. Additionally, test soils should be characterized by:

— texture (sand, silt, clay);

— pH value;

— salinity;

— organic carbon;

— total and water-soluble amounts of potassium, nitrogen and phosphorus.

Soil pH should not be corrected. Measurements of soil contaminants (heavy metals, hydrocarbons, pesticides, explosives, PCBs and others) are not mandatory.

It should be checked whether the test soil sucks water via wicks sufficiently (see 5.4). Water repellency or poor water transport can occur with very sandy soils, soils highly contaminated with hydrocarbons or even with soils of high clay content that tend to compact even when these soils have a high water-holding capacity (determined after initially submerging the soils). To assure functioning of the watering system, a pre-test including all soils selected for the test and replicated twice should be performed to decide whether wick watering is sufficient or manual watering is required.

NOTE For the time being, pH limits for plant species other than turnip rape and oat cannot be given. It is matter of future research to systematically test more plants on a variety of soils. Furthermore, tolerance limits for texture, salinity or other soil properties cannot yet be given for different plant species.

5.2.3 Control soil

Either artificial, reference or standard soils may be used as control substrate.

If reference soils from uncontaminated areas near a contaminated site are available, they should be treated and characterized like the test soils. In addition, to verify that a reference soil does not carry toxic contaminants, chemical analysis of the expected contaminants shall be carried out. If toxic contamination or unusual soil properties cannot be ruled out, standard control soils should be given preference.

Standard soils should be uncontaminated, nutrient-poor natural or artificial soils. If a natural soil is used, its organic matter content should not exceed 5 %. Fine particles (< 20 µm) should not exceed 20 %.

Alternatively, artificial soil in accordance with reference [6] and ISO 11268-2 may be used, regardless of its higher organic matter content. However, the organic matter contents of the test and control soil should be as close to each other as possible. The artificial soil consists of the following components (percentage based on dry mass):

— 10 % sphagnum peat [air-dried and finely ground (2 mm ± 1 mm)];

— 20 % kaolin clay (kaolinite content preferably above 30 %);

— approximately 69 % (depending on the amount of $CaCO_3$ needed) air-dried industrial quartz sand (predominantly fine sand with more than 50 % mass fraction of particle size 0,05 mm to 0,2 mm).