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Soil quality — Determination of the toxic effects of pollutants on germination and early growth of higher plants

Qualité du sol — Détermination des effets toxiques des polluants sur la germination et la croissance primaire des plantes supérieures

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Foreword

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

Ecotoxicological testing of test soils or waste materials to be disposed on soil are required to assess the potential environmental risk resulting from soil pollution or the disposal of wastes such as sewage sludge on farmland. There is also a need to monitor the quality of soil after reclamation of industrial sites. Therefore, a very practical and rapid germination and growth test has been developed based on seed germination and seedling growth in controlled environmental conditions.

The assay, which does not require any pretreatment of the seeds, is performed in "transparent test plates", incubated vertically, to allow the roots and the shoots of the germinated seeds to be seen. After 72 h exposure, a picture of the transparent test plates is taken and can be analysed "by image analysis" for multiple endpoints, such as percentage of seed germination and of length of roots and shoots. To account for the plant species variability in sensitivity, the assays are performed with the seeds of three plant species: one monocotyl (*Sorghum saccharatum*) and two dicotyls (*Lepidium sativum* and *Sinapis alba*).

A major advantage of this test is that after the shooting and storing of the pictures of the test plates, the measurements by image analysis can be postponed to any appropriate timing.

Reference or standard soils can be used as negative controls, such as, for example, the ISO standard artificial soil according to ISO 11269-1 and ISO 11269-2.

Commercially available seeds, with a shelf life longer than one year, allow the use of this test at any time of the year.

Two International interlaboratory comparisons demonstrated that the test provides good results.

A substantial number of studies report data on the application of this test on various types of soils and soil materials with several types of plant species.

Soil quality — Determination of the toxic effects of pollutants on germination and early growth of higher plants

1 Scope

This International Standard describes a technique for determining the effects of soil and soil-related materials on the seed germination and early growth of higher plants. These endpoints are useful indicators for the assessment of the quality of a soil as a habitat for organisms. This International Standard is applicable to all soils in which soil organisms are active and may be used to evaluate:

— the effects on plants due to toxicity of solid or liquid chemicals contaminating soil or materials (compost, sludge, waste) and chemicals added to soil;

— the changes in the soil effect on plants after restoration measures.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 11269-1, *Soil quality — Determination of the effects of pollutants on soil flora — Part 1: Method for the measurement of inhibition of root growth*

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

ISO/TS 20281, *Water quality — Guidance on statistical interpretation of ecotoxicity data*

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 prepared according to ISO 11269-1 and ISO 11269-2

3.2
control soil
reference or standard soil used as a control and as a medium for preparing dilution series with test soils or a reference substance

3.3
reference soil
uncontaminated site-specific soil (e.g. collected in the vicinity of a contaminated site) with similar properties (nutrient concentrations, pH, organic carbon content and texture) as the test soil

3.4
standard soil
field-collected soil or artificial soil whose main properties (e.g. pH, texture, organic matter content) are within a known range

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

EXAMPLE Euro-soils[1], artificial soil[2], LUFA soil.[1)]

3.5
test soil
either a natural or an artificial clean soil that is spiked with the test substance or a contaminated natural soil (a site soil)[5]

3.6
seeding emergence
appearance of a visible seedling above the surface of the cover material

[SOURCE: ISO 17126:2005, 3.1, modified]

3.7
germination
appearance of a root of at least 1 mm of length

3.8
pure water
grade of water, produced, for example, by single distillation, by de-ionization, by ultra-filtration or by reverse osmosis[5]

3.9
root length
length of the root from seed to root tip

3.10
shoot length
length of the part that grows upward, from seed to tip

3.11
water saturation
maximum water content that a soil can retain against gravity under undisturbed soil conditions, conventionally stated as water content two days to three days after full saturation with water

[SOURCE: ISO 11074:2015, 2.1.5 field capacity, modified]

3.12
water saturated soil
soil which has reached its maximum water content

3.13
water-holding capacity
mass of water that evaporates from soil saturated with water when the soil is dried to constant mass at 105 °C, divided by the dry mass of the soil[7]

3.14
negative control
any well-characterized material or substance that, when tested by a specific procedure, demonstrates the suitability of the procedure to yield a reproducible, appropriately negative, non-reactive or minimal response in the test system

[SOURCE: ISO 10993-10:2010, 3.12, modified]

3.15
effect percentage
percentage decrease of the seed germination and the growth of the plant roots and/or shoots in the test soil in comparison to the control soil

1) Euro-soils, artificial soil and LUFA soil are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

4 Principle

This method compares the seed germination and early growth of monocotyledonous and dicotyledonous plants in a test soil and/or a series of mixtures with a control soil. This method may also be used for the testing of compost, sludge or waste.

Seeds of one monocotyledonous plant, such as *Sorghum saccharatum* (L.) Moench, and two dicotyledonous plants, such as *Lepidium sativum* L. and *Sinapis alba* L., are exposed to the test material under controlled conditions. After (72 ± 1) h, the number of germinated seeds is recorded and the length of the roots of the test plants is measured in the test soil and in the control soil.

If different seed species are used, the length of the incubation period may be adjusted, depending on the time of germination of the seeds and the growth speed of the roots.

The test makes use of unique flat and shallow transparent test plates (6.3) composed of two compartments, the lower one of which contains hydrated soil.

Seeds of the selected test plants are positioned at equal distance near the middle ridge of the test plate (6.3) on a black filter paper (6.5) placed on top of the hydrated soil.

After closing the test plates (6.3) with their transparent cover, the test plates are placed vertically in a holder (6.4) and incubated at (25 ± 1) °C for (72 ± 1) h.

At the end of the incubation period, the length of each root (and shoot, if wished) can be measured directly with a ruler and recorded.

Alternatively, a "digital" picture is taken of the test plates (6.3) with the germinated plants (either with a digital camera, a webcam camera or a flatbed paper scanner) for storage in a computer file. The subsequent root length measurements are performed by image analysis. The analyses on germination and root growth can then be made immediately or postponed to any appropriate timing.

NOTE The same procedure can be applied to also measure the shoot height, if wished. Calculation of the shoot/root length ratio is a possible additional effect parameter.

5 Reagents, test organisms and media

5.1 Water.

Pure water having a conductivity below 10 µS/cm.

5.2 Test organisms.

The test organisms are seeds of one monocotyledonous plant, such as *Sorghum saccharatum* (L.) Moench, and two dicotyledonous plants, such as *Lepidium sativum* L. and *Sinapis alba* L.

Investigations have been performed not only with the three plant species indicated in 5.2 but also with other monocotyl and dicotyl plant species. A synthesis on these published studies is given in Annex A.

Seeds coated with insecticides and/or fungicides should be avoided.

5.3 Control soil.

Either reference or standard soils can be used as the control soil, if unhindered growth of the test plants in these soils can be expected.

When comparing the root elongation in soils of known and unknown quality, the control soil and soil under test should be of the same textural class, and be as similar as practicable in all respects other than the presence of the chemical or contaminant being investigated. Indeed, significant differences in soil characteristics other than the presence of contaminant may lead to differences in root lengths and may induce false positive test results.

Alternatively, artificial soil according to ISO 11269-1 and ISO 11269-2 may be used. The substrate called "artificial soil" has the following composition:

	Percentage expressed on dry-mass basis
Sphagnum peat finely ground and with no visible plant remains	10 %
Kaolinite clay containing not less than 30 % kaolinite	20 %
Industrial quartz sand (dominant fine sand with more than 50 % of particle size 0,05 mm to 0,2 mm)	69 %

NOTE As indicated in ISO 11269-1, 5 % peat have proven to be sufficient for maintaining the desired structure of the artificial soil (with a corresponding increase of the sand percentage to 75 %). A lower percentage of kaolinite clay (10 % instead of 20 %) is furthermore very close to the clay content of LUFA 2.2 and hence more representative for a natural soil. The following composition of the artificial soil is therefore recommended: peat 5 %, kaolinite clay 10 % and sand 85 %.

Approximately 0,3 % to 1,0 % calcium carbonate ($CaCO_3$, pulverized, analytical grade) is necessary to get a pH of 6,0 ± 0,5.

6 Apparatus and materials

6.1 Incubator or temperature controlled room, suitable for maintaining the specified conditions at (25 ± 1) °C.

6.2 Digital camera, webcam camera or flatbed paper scanner, to shoot pictures of the test plates with the germinated seeds, for storage in a computer file.

6.3 Test plates, transparent plates in polyvinylchloride (PVC).

The test plates are composed of a bottom part separated by a middle ridge into an upper part and a lower part and a flat cover. Test plates can be "handmade" with the aid of transparent PVC sheet and small rectangular sticks as described in Annex B.

Alternatively, commercially available[2)] test plates can be used. The lower part of these test plates is intended to hold approximately 90 cm^3 of test soil. The latter test plates have on both parts on their side small rectangular cavities for closing the plates tightly by a unique click system. The test plates shall be provided with a label to record the specifics of each test plate (type of soil, type of seed, number of the replicate).

2) The test plates supplied by MicroBioTests Inc. Mariakerke-Gent, Belgium are an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

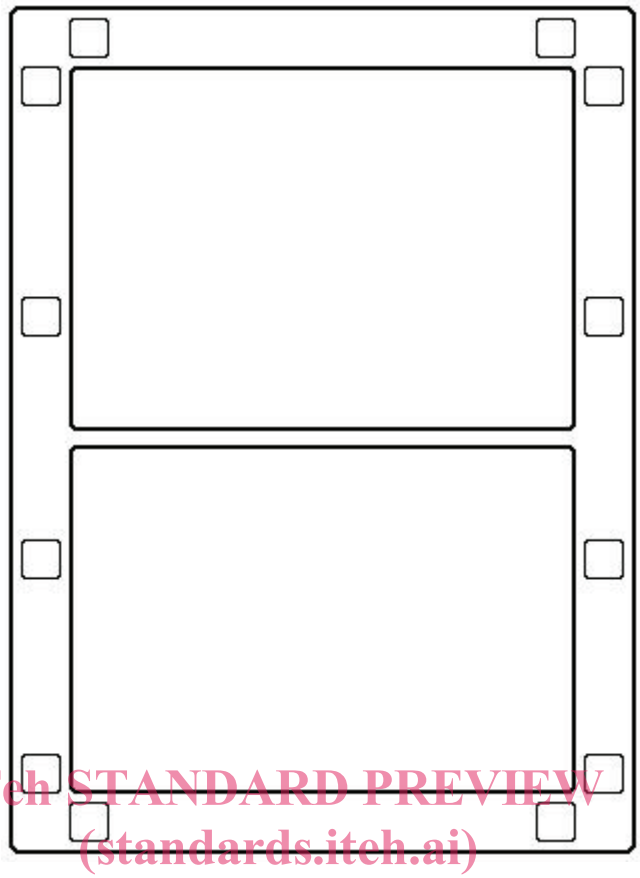

Figure 1 — Test plate with cavities on the side for tight closing of the plate

6.4 Test plate holders, cardboard holders for vertical incubation of six test plates (6.3) each.

6.5 Black filter papers, rectangular high purity black filter papers (e.g. 85 g/m^2, 0,17 mm thickness, 45 s filtration speed) fitting the lower part of the test plate, to be placed on top of the soil in the lower compartment of the test plates (6.3).

6.6 Microsieve cylinder, small plastic cylinder provided at the bottom with a nylon gauze, to be used for determination of the water to be added to the test soil.

6.7 Wide mouth micropipette, plastic micropipette to be used with the microsieve cylinder (6.6) for determination of the water to be added to the test soil.

6.8 Sieve, sieve of 2 mm mesh for sieving the test soil prior to use for the tests.

6.9 Thin spatula, hand tool with a thin blade used to mix the test soil with water.

6.10 Flat spatula, hand tool with a broad, flat blade that is used to spread and flatten the test soil into the lower compartment of the test plates (6.3).

6.11 Tweezers, small pincer-like tool for handling the seeds.

7 Treatment and preparation of samples

7.1 Soil samples

The assays are carried out at water saturation of the soils at the start of the tests.

7.1.1 Water-holding capacity determination

The water saturated soil can be obtained by adding to the soil mass the appropriate volume of pure water determining the water-holding capacity.

7.1.2 Alternative procedure for determination of the volume of water to be added in the test plates for hydration of air-dried soils

A simple and quick alternative procedure can be used to determine the volume of water to be added to air-dried soils in the test plates (6.3).

For the artificial soil recommended in 5.3, the amount of water needed to be added for hydration has been determined experimentally. Based on a water/soil ratio (on a vol/vol basis) of 0,39, one needs to add 35 ml pure water to the 90 cm^3 control soil in the test plate (6.3).

For other control soils, the amount of water needed to be added for hydration needs to be experimentally determined, following the procedure indicated in the next paragraph for test soil samples.

For test soil samples, they first need to be air-dried, then the dry soil is sieved through a sieve (6.8) to eliminate all coarse material.

NOTE Air drying is requested only on a separate soil sample for subsequent determination of the volume of water to be added in the test plates.

Mix thoroughly 50 ml pure water and 90 cm^3 test soil in a beaker with the aid of a thin spatula (6.9). After 1 min to 2 min, two layers show up: the hydrated soil and a layer of water on top.

Lower vertically the microsieve cylinder (6.6) into the beaker, down to the surface of the hydrated soil, and then lower it a little further down, so that it starts filling with supernatant.

With the wide mouth micropipette (6.7), suck up the water inside the microsieve and transfer it into a graduated cylinder.

Put the microsieve cylinder (6.6) again into the beaker, and push it down a little further, so that it takes up additional water from the soil. Transfer again the recovered water in the graduated cylinder and repeat the former manipulations until no water comes out anymore from the soil.

Calculate how much water was needed for a complete hydration of the test soil. This volume (Vsat) is the volume of water that has originally been added to the soil (= 50 ml) minus the volume of supernatant water (S) which has been recovered in the graduated cylinder (Vsat = 50 − S).

8 Procedure

The procedure described hereafter is intended for use of 90 cm^3 soil in the test plates (6.3).

In case of assessment of the dose-response relationship on an unknown solid sample, a full test shall be performed comprising a number of mixtures of the control soil with the sample (e.g. compost, sludge, waste). The dilutions shall be prepared within a geometric series with a separation factor not exceeding 2,0 (see ISO 11269-1). According to the selected dilution range, the test soil is mixed with the reference soil or the standard soil thoroughly (either manually or by using a hand mixer). The homogeneity of the mixture is checked visually. For each of the resulting dilutions, the volume of water to be added in the test plates (6.3) shall be experimentally determined (7.1.2).

