SLOVENSKI STANDARD
oSIST prEN ISO 18763:2020

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Kakovost tal - Določevanje vpliva onesnaževal na kalivost in zgodnjo rast višjih rastlin (ISO 18763:2016)

Soil quality - Determination of the toxic effects of pollutants on germination and early growth of higher plants (ISO 18763:2016)

Qualité du sol - Détermination des effets toxiques des polluants sur la germination et la croissance primaire des plantes supérieures (ISO 18763:2016)

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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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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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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.