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Soil quality — Determination of the effects of pollutants on soil flora —

Part 1: Method for the measurement of inhibition of root growth

Qualité du sol — Détermination des effets des polluants sur la flore du sol —

Partie 1: Méthode de mesurage de l'inhibition de la croissance des racines

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 11269-1 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 11269-1:1993), which has been technically revised.

ISO 11269 consists of the following parts, under the general title *Soil quality — Determination of the effects of pollutants on soil flora*:

— *Part 1: Method for the measurement of inhibition of root growth*

— *Part 2: Effects of contaminated soil on the emergence and early growth of higher plants*

Introduction

Chemical analysis of soil samples or waste materials to be disposed on soil, together with ecotoxicological testing, provides substantial evidence of the suitability of the soil for arable production, or gives information on the potential environmental risk resulting from the disposal of wastes such as sewage sludge on farmland. There is also a need to assess the quality of the soil after reclamation of industrial sites and colliery tips or when capping landfill sites. As the ability of the soil to grow crops is the main criterion, a rapid-growth test has been developed, based on seedling growth in controlled environmental conditions.

Two major prerequisites of a phytotoxicity test are that it provides consistently reliable results and that it can be used at any time of the year. It is therefore essential that seeds be grown in a controlled environment to ensure optimal growing conditions which can be maintained for any number of tests, producing reproducible results over a long period of time.

The test method described in this part of ISO 11269 can be used to compare soils, to monitor changes in their activity or to determine the effect of added chemicals or materials (compost, sludge, waste).

Soil quality — Determination of the effects of pollutants on soil flora —

Part 1: Method for the measurement of inhibition of root growth

1 Scope

This part of ISO 11269 describes a method for the determination of the effects of contaminated soils or contaminated samples on the root elongation of terrestrial plants.

This method is applicable to soils, soil materials, compost, sludge, waste or chemical testing. It is applicable to the comparison of soils of known and unknown quality and to the measurement of effects of materials (compost, sludge, waste) or chemicals deliberately added to the soil.

The method is not intended to be used as a measure of the ability of the soil to support sustained plant growth.

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 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

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

ISO 10930, *Soil quality — Measurement of the stability of soil aggregates subjected to the action of water*

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

ISO 11268-1, *Soil quality — Effects of pollutants on earthworms — Part 1: Determination of acute toxicity to Eisenia fetida/Eisenia andrei*

ISO 11268-2, *Soil quality — Effects of pollutants on earthworms — Part 2: Determination of effects on reproduction to Eisenia fetida/Eisenia andrei*

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

3.2
test mixture
mixture of test soil or test material (compost, sludge, waste or chemical) with control soil

3.3
radicle
portion of the plant embryo which develops into the primary root

3.4
hypocotyl
portion of the axis of an embryo or seedling situated between the cotyledons (seed leaves) and the radicle

3.5
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

3.6
standard soil
field-collected soil or artificial soil whose main properties (e.g. pH, texture, organic matter content) are within a known range

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

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

3.7
control soil
reference or standard soil used as a control and as a medium for preparing dilution series with test soil or test material (e.g. compost, sludge, waste, chemical)

NOTE Both the effective concentration (EC_x) and the no-observed-effect concentration (NOEC) are expressed in milligrams of test substance per kilogram (dry mass) of the test substrate. Soil mixtures are given in percent based on soil dry mass.

3.8
effective concentration
EC_x
effective concentration (dilution) of the test soil or test material (e.g. compost, sludge, waste, chemical) at which root elongation is reduced by x % compared to the control

4 Principle

This method compares the root elongation of terrestrial plants in a test soil and/or a series of dilutions with a control soil. This method may also be used for the testing of compost, sludge, waste or chemicals by applying various concentrations of the material under investigation to a control soil.

Pregerminated seeds are exposed to the test material under controlled conditions. After the growth period, the lengths of the roots of the test plants are compared with those of the control plants. Statistically significant differences in the root lengths of seedlings grown in any test medium compared to the controls are indicative of an effect.

NOTE Shoot height is also a useful parameter, and this can be measured in conjunction with root length to provide additional or corroborative data.

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.

5 Test plants

Winter barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and wheat (*Triticum aestivum* L.) are the recommended species. Other monocotyledonous plant species might be selected, e.g. plants with ecological or economic significance in certain regions of the world, provided that the roots of these plants grow unhindered in sand and in control soil under the conditions specified. Only plants that tolerate the properties of the test soils and test conditions (besides 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.

Seeds coated with insecticides and/or fungicides should not be used.

NOTE The methodology of this test can also be adapted for use with dicotyledonous species with straight roots, which are easily measurable.

6 Materials

6.1 Test vessels

The test vessels shall be cylindrical, at least 8 cm in diameter and 11 cm in height, and shall have parallel sides to ensure that the roots of seedlings are not restricted and do not encounter tapering side walls. The base of the pots shall be perforated and covered with filter paper.

NOTE When filled to a height of 10 cm, the pots contain approximately 500 g of sand, 400 g of air-dried soil and 250 g of artificial soil.

6.2 Soil

6.2.1 Test soil

Some physical characteristics of the test soil can induce disturbances in root elongation such as heterogeneous soil with big particles or clayey soil with a high water content. Therefore, the soil to be tested shall be passed through a sieve with a 2 mm square mesh to remove coarse fragments. Furthermore, fine particles (<20 µm according to ISO 11277) should not exceed 20 % of the dry mass.

Before the test, the soil is stored in accordance with ISO 10381-6.

For each soil, the following characteristics should be determined:

a) soil texture classification;

b) pH (KCl) in accordance with ISO 10390;

c) water content in accordance with ISO 11465;

d) water-holding capacity in accordance with Annex A;

e) cationic exchange capacity in accordance with ISO 11260;

f) organic matter content in accordance with ISO 10694.

6.2.2 Control soil

Either reference or standard soils may be used as the control soil.

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. 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.

6.2.2.1 Reference soil

If a reference soil from an uncontaminated area near a contaminated site is available, it should be treated and characterized like the test soil (6.2.1). If a toxic contamination or unusual soil properties cannot be ruled out, a standard control soil should be preferred.

6.2.2.2 Standard soil

The standard soil should be uncontaminated, nutrient-poor natural or artificial soil. If a natural soil is used, its organic matter content should not exceed 5 %. Fine particles (<20 µm according to ISO 11277) should not exceed 20 %.

Alternatively, artificial soil in accordance with ISO 11268-1 and ISO 11268-2 may be used, regardless of its higher organic matter content. However, the organic matter content of the test and control soil should be as close to each other as possible.

NOTE 1 Artificial soil may be used to assess the effects of compost, sludge, waste or chemicals deliberately added to the soil.

The substrate called “artificial soil” has the following composition (Table 1).

Table 1 — Constituents of the artificial soil

Constituents	Percentage expressed on a dry-mass basis
Sphagnum peat, air-dried, 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 between 0,05 mm and 0,20 mm)	70 %

Calcium carbonate ($CaCO_3$, pulverized, analytical grade) is necessary to bring the pH (KCl) of the wetted substrate to 6,0 ± 0,5 (commonly between 0,3 % and 1,0 % of the mass of the dry ingredients).

NOTE 2 Taking the properties of highly non-polar (log $P_{O/W} > 2$) or ionizing substances into account, 5 % of peat have proven to be sufficient for maintaining the desired structure of the artificial soil. In this case, the respective percentages of the constituents are modified as follows: peat, 5 %; clay, 20 %; sand 75 %.

The artificial soil is prepared by thoroughly mixing the dry constituents listed above in a large-scale laboratory mixer (7.4). The amount of calcium carbonate required might vary, depending on the properties of the individual batch (mainly the peat), and should be determined by measuring subsamples immediately before the test.

The mixed artificial soil is stored at room temperature. To determine the pH and the total water-holding capacity, the dry artificial soil is premoistened at least two days before starting the test by adding deionized water to obtain half of the required final water content of 70 % ± 5 % of the total water-holding capacity. The water-holding capacity and pH (KCl) are determined in accordance with Annex A and ISO 10390 respectively. If the measured pH is not within the required range, a sufficient amount of $CaCO_3$ shall be added or a new batch of artificial soil shall be prepared.

6.2.3 Sand control

In order to demonstrate the uniformity of the laboratory test conditions, three pots, filled with sand, are included in each root-growth inhibition test.

The root length is related to the species and varieties used but also to the growing conditions. Table 2 provides examples of results of sand control obtained with the three recommended species. Laboratories should establish a control chart for each selected strain. Once sufficient data are available (i.e. 10 values of root elongation in sand control), the acceptable range (mean value ± 2s where s is the standard deviation) is calculated and used to determine whether the results are within ±2s of the respective values obtained in previous tests. The acceptable range is updated with new data obtained from the sand control.

Table 2 — Results of sand control obtained with the three recommended species

Species	Number of tests	Min. – max. values	Mean	Mean ± 2*s*
Winter barley (variety "platine")	12	112,7 – 146,6	131,9	114,2 – 149,7
Oat (variety "fringante")	9	97,8 – 119,0	112,8	100,4 – 124,8
Wheat (variety "Rosario")	10	84,0 – 109,9	91,4	77,8 – 105,7

The sand is a washed industrial sand or other similar pure sand of the following particle-size distribution: 10 % > 0,6 mm, 80 % between 0,2 mm and 0,6 mm, 10 % < 0,2 mm.

NOTE Sand may also be used to assess the effects of compost, sludge, waste or chemicals deliberately added to the soil, as an alternative to control soil.

7 Equipment

Standard laboratory equipment including the following.

7.1 Controlled environmental chamber, phytotron, plant-growth room or greenhouse, suitable for maintaining the specified conditions.

7.2 Balance, with an accuracy of 0,1 g.

7.3 Resealable polyethylene bags (36 cm × 18 cm).

7.4 Large-scale laboratory mixer, for the preparation of the artificial soil.

7.5 Sieve, stainless steel, with a mesh size of 2 mm.

8 Reference substance

It is recommended that a test be carried out regularly with a reference chemical in order to demonstrate the uniformity of the laboratory test conditions. Nickel sulfate ($NiSO_4$ $6H_2O$) and boric acid (H_3BO_3) are suitable reference substances.

Reference data are presented in Annexes B and C.

9 Procedure

9.1 Experimental design

According to the aim of the study, the experimental design is either a limit test (e.g. comparison of soil of known and unknown quality, disposal of sewage sludge on farmland) or a full test (assessment of dose–response relationship). For the latter, a preliminary test may be performed to determine the range of mixture ratios affecting root elongation. This preliminary test is conducted without replication.

The full test shall comprise at least five test mixtures or concentrations selected within a geometric series with a separation factor not exceeding 2,0.

The pots containing the sand, the control soil, the soil to be tested and/or the series of test mixtures (soil/compost, sludge or waste or soil/chemical) shall be replicated three times.