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ISO 11269-2

Third edition
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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

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

Partie 2: Effets des sols contaminés sur l'émergence et la croissance des végétaux supérieurs

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Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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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 11269-2 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

This third edition cancels and replaces the second edition (ISO 11269-2:2005), 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*:

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— *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*

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Introduction

This part of ISO 11269 describes a procedure for evaluating the quality of soils of different origin carrying unknown contaminations. The evaluation of the effects on plant growth is based on emergence and inhibitory effects on early growth of at least two species of higher plants. Guidance for assessing potential effects of substances on seedling emergence and growth is given in OECD Guideline 208[14].

This part of ISO 11269 refers closely to ISO 22030 and is based on:

a) results from the German research project "Entwicklung eines innovativen und technischen Instrumentariums zur Optimierung der ökotoxikologischen Bewertung von Böden im Hinblick auf Sanierungsziele und Schutzerfordernisse";

b) discussions within the joint project "Ecotoxicological Test Batteries" forming part of the BMBF Joint Research Group "Processes for the Bioremediation of Soil"[23];

c) results from the BMBF Joint Research Group ERNTE "Erprobung und Vorbereitung einer praktischen Nutzung ökotoxikologischer Testsysteme"[17];

d) ring-test results of "Ecotoxicological Characterisation of Waste — Results and Experiences from an International Ring Test"[8].

Plant growth can be influenced strongly by soil properties such as texture, pH or levels of nutrients. When testing natural soils either reference soils (uncontaminated soils with the same properties as the test soil) or standard soils are used as mixing and control substrate. In the latter case, variations in plant growth can result from either soil contaminants or differences in soil properties like nutrients and texture. Therefore, results from soil testing can less easily be interpreted than results from testing of chemicals .

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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

WARNING — Contaminated soils may contain unknown mixtures of toxic, mutagenic, or otherwise harmful chemicals or infectious micro-organisms. Occupational health risks may arise from dust or evaporated chemicals during handling and incubation. Furthermore, test plants might take up chemicals from the soil and safety measures should also be considered when handling the test plants.

1 Scope

This part of ISO 11269 describes a method to assess the quality of an unknown soil and the soil habitat function by determining the emergence and early growth response of at least two terrestrial plant species compared to reference or standard control soils. It is applicable to soils of unknown quality, e.g. from contaminated sites, amended soils or soils after remediation.

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2 Normative references

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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 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 22030, *Soil quality — Biological methods — Chronic toxicity in higher plants*

3 Terms and definitions

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

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3.1
emergence
appearance of the coleoptile or cotyledon above the soil

3.2
contaminant
substance or agent present in the soil as a result of human activity

[ISO 15176:2002[28]]

3.3
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][2]

3.4
lowest observed effect rate or effect concentration
LOEC
lowest tested percentage of a test soil in a reference or a standard control soil or concentration of a substance at which a statistically significant effect is observed

NOTE The LOEC is expressed as a percentage of the test-soil dry mass per soil-mixture dry mass. All test mixtures above the LOEC have a harmful effect equal to 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 (3.5) have been selected.

3.5
no observed effect concentration
NOEC
test-soil percentage immediately below the LOEC, which when compared to the control has no statistically significant effect ($\rho < 0{,}05$)

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3.6
***x* % effect concentration**
EC_x
***x* % effect rate**
ER_x
percentage of a test soil at which a given endpoint is inhibited by x % compared to the control

3.7
soil mixture ratio
ratio between the test soil and the reference/control soil in a soil mixture, expressed in percent based on soil dry mass

NOTE Different ratios may be applied in a dilution series to establish a dose-response relationship.

3.8
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

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3.9
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[11], artificial soil[14], LUFA soil.[1)]

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

3.10
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

NOTE Both EC_{50} and NOEC are expressed in milligrams of test substance per kilogram (dry mass) of the test substrate. Soil mixtures are given in per cent based on soil dry mass.

4 Units

Emergence is expressed as the percentage of seedlings which emerge as compared with the control pots. The biomass of the shoots is expressed as dry mass per plant or, if needed, as dry mass per pot.

5 Principle

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The test measures emergence and early growth of at least two terrestrial plant species (one monocotyledonous and one dicotyledonous). The test compares the development of plants in a test soil and/or a series of mixtures with a control soil. Seeds of the selected plant species are planted in pots containing the soil/soil mixtures and in control pots containing a reference or standard soil. Pots are kept under growth conditions for the test species selected. After 50 % of the seedlings in the control have emerged, emergence rates are determined and plants are thinned out to a specified number. After a period of two weeks to three weeks, the remaining plants are harvested to determine their biomass. The relative growth inhibition in undiluted test soil is determined to assess the function of the test soil as a habitat for plants. In addition, NOEC, LOEC or EC_x and ER_x values can be calculated from the dose response curve gained from mixtures of the test soil with control soil.

NOTE An early plant growth test may include additional testing endpoints, e.g. shoot length, root length and root dry mass. In many instances, root endpoints are more sensitive than shoot dry mass. In almost all cases, emergence is a less sensitive endpoint.

6 Test plants

One monocotyledonous and one dicotyledonous species are tested in parallel. Oat (*Avena sativa*) is recommended as the monocotyledonous and turnip rape (*Brassica rapa*) and/or wild turnip (*Brassica rapa* ssp. *rapa*) as the dicotyledonous plant species. Oat, turnip rape and wild turnip grow in sandy as well as in loamy soil with varying water content and a range of pH values from 5,0 to 7,5.

Other species might be selected, e.g. plants with specific physiological characteristics like C-4 plants (corn, sugar cane, millet), plants in symbiosis with nitrogen-fixing bacteria (e.g. Fabaceae) or plants with ecological or economical significance in certain regions of the world, provided that these species grow unhindered in control soil and fulfil the validity criteria of the test (Clause 11). Only plants that tolerate the properties of the test soils and test conditions (beside 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. Species that do not tolerate wet soils should not be used in combination with wick watering. Reasons for selecting species other than oat and wild turnip or turnip rape shall be justified in the test report.

NOTE Additional recommended species including validity criteria and reference toxicant test data for different endpoints are compiled in Annexes A and B.

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.

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7 Materials

7.1 Test vessels

The test vessels shall be non-porous plastics or glazed pots with a top internal diameter of between 85 mm and 95 mm, taking into account the size. It is recommended to use an automatic watering system, e.g. pots equipped with glass-fibre wicks, to avoid the time-consuming daily manual adjustment of soil moisture as proposed in ISO 22030. In this case, one or two glass-fibre wicks (∅ 1 mm) shall be introduced through the bottom of the vessels. The wicks reach a water reservoir and ensure the water supply during the test. Therefore, at least one hole shall be prepared to fix the wick. Commercial plant pots often have more than one hole, what might result in flow back of water. In addition, roots might grow through open holes and circumvent the soil contaminants. A filter disk can prevent growth of roots through additional holes. Wicks are not used, when the test soil does not take up water by wicks as shown by a pretest (see 10.2).

7.2 Soil

7.2.1 General

Assessing the toxic potential of field soil from a contaminated site or that of remediated soil, 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*.

The soil pH should not be corrected. For the time being, pH limits for plant species other than turnip rape and oat cannot be stated. It is a 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 be given for different plant species so far.

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When comparing soils of known and unknown quality, the control soil and field 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 contaminants may lead to differences in plant growth and may induce false positive test results.

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7.2.2 Test soil

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

The soils used in the test shall be passed through a sieve of 4 mm square mesh to remove coarse fragments and thoroughly mixed. If necessary, soil may be air dried without heating before sieving. Storage of test soils should be as short as possible. The soil shall be stored in accordance with ISO 10381-6 using containers that minimize losses of soil contaminants by volatilization and sorption to the container walls. Soil pH should not be corrected as it may influence bioavailability of soil contaminants.

For interpretation of test results, the following characteristics should be determined for each soil sampled from a field site:

a) soil texture (sand, loam, silt) in accordance with ISO 11277,

b) pH (KCl) value in accordance with ISO 10390,

c) water content in accordance with ISO 11465,

d) water-holding capacity (Annex C),

e) cationic exchange capacity in accordance with ISO 11260,

f) organic matter content in accordance with ISO 10694,

g) total and water soluble amounts of potassium, nitrogen and phosphorous.

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

7.2.3 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. 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 might 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 (3.3) can be expected at low soil mixture ratios or even an inverse dose response relationship if nutrient supply becomes the main effect. It is therefore recommended to add nutrients to test and control/reference soils in order to avoid false positive or negative test results (10.6.3).

7.2.3.1 Reference soils

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.

7.2.3.2 Standard soils

Standard soils 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 according to ISO11268-1 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.

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The substrate called “artificial soil” has the following composition:

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	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 %

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

NOTE 1 Taking the properties of non-polar (log $P_{OW} > 2$) 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 %).

NOTE 2 pH (KCl) is measured in a mixed sample in a 1 M solution of potassium chloride (KCl) or a 0,01 M solution of calcium chloride ($CaCl_2$).

The artificial soil is prepared, at least three days prior to starting the test, by mixing the dry constituents listed above thoroughly in a large-scale laboratory mixer. A portion of the deionized water required to obtain half of the final water content of 40 % to 60 % of the maximum water-holding capacity is added while mixing is continued. The mixed artificial soil shall be stored at room temperature for at least two days to equilibrate acidity. The amount of calcium carbonate required might vary, depending on properties of the individual batch of sphagnum peat and should be determined by measuring the pH of subsamples immediately before the test. The total water-holding capacity is determined according to Annex C, the pH is determined according to ISO 10390.

Allowance should be made for any water that is to be used for introducing the test substance into the soil.

To obtain a dilution series, the test soil is mixed with the control soil thoroughly (either manually or by using a hand mixer). The homogeneity of the mixture is checked visually.

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