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

Qualité du sol -- Détermination des effets des polluants sur la flore du sol -- Partie 2: Effets des substances chimiques sur l'émergence et la croissance des végétaux supérieurs

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INTERNATIONAL STANDARD

ISO 11269-2

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

Part 2:
Effects of chemicals on the emergence and growth of higher plants

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

Partie 2: Effets des substances chimiques sur l'émergence et la croissance des végétaux supérieurs

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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.

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.

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

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 chemicals on the emergence and growth of higher plants*
- *Part 3: Method for the measurement of germination*
- *Part 4: Guidance on field testing using higher plants*

Annexes A, B and C of this part of ISO 11269 are for information only.

Introduction

This part of ISO 11269 describes a method for the evaluation of soil quality following the addition of chemicals or after contamination of an unknown type. Before assessing the effects on plant growth of a chemical incorporated in soil, information on the solubility in water and in organic solvents, and the vapour pressure of the test substance is required. Preferably, the molecular formula, partition coefficient (water:octanol), and chemical and biological stability should be available to the laboratory. All physical and biological parameters should be considered when interpreting the results of the test.

The test, as written, assesses the effect on emergence and plant growth of a chemical incorporated in soil. In the case of contaminated soil, the individual chemicals are unidentified and therefore correct information on solubility, vapour pressure and molecular formula etc. cannot be selected. No incorporation is required, but it may be necessary to dilute with uncontaminated control soil or sand before testing.

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

Part 2:
Effects of chemicals on the emergence and growth of higher plants

1 Scope

This part of ISO 11269 describes a method that is applicable to the determination of possible toxic effects of solid or liquid chemicals incorporated in soil on the emergence and early stages of growth and development of a variety of terrestrial plants. It does not give an indication of damage resulting from direct contact of seedlings with the chemical in the vapour or liquid phase outside the soil environment.

The method is also applicable to the comparison of soils of known and unknown quality. Information on how to adapt the method for this purpose is given in annex B.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 11269. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 11269 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10381-6:1993, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory.*

ISO 10390:1994, *Soil quality — Determination of pH.*

3 Definitions

For the purposes of this part of ISO 11269, the following definitions apply.

3.1 LOEC (lowest observed effect concentration): Lowest concentration used in the test at which the substance is observed to have a significant effect on emergence or growth as compared with the control. All test concentrations above the LOEC shall have a harmful effect equal to or greater than those observed at the LOEC.

3.2 NOEC (no observed effect concentration): Test concentration immediately below the LOEC.

3.3 visual assessment: Describes any damage to the test species, in terms of stunting, chlorosis or necrosis in both treated and control pots.

4 Units

Concentrations of test substances are expressed as milligrams per kilogram of dry soil.

Emergence is expressed as the percentage of seedlings which emerge compared with the control pots.

Effects on growth are expressed as the difference in mass of the aerial parts of the plants between the treated plants and those in the control pots.

5 Principle

This phytotoxicity test is based on the emergence and early growth response of a variety of terrestrial plant species to various concentrations of a chemical added to the test soil.

Seeds of selected species of plants are planted in pots containing soil to which the test chemical has been added and in control pots. The pots are kept under growth conditions for the test species selected. The emergence and mass (dry or fresh basis) of the shoots of the test plants are compared with those of the control plants.

6 Test plants and materials

6.1 Equipment

Suitable facilities for carrying out the test include phytotrons, plant growth rooms and greenhouses. The planting containers shall be non-porous plastics or glazed pots with a top internal diameter of between 85 mm and 95 mm.

6.2 Test plants

A minimum of two species shall be selected for the test, comprising at least one species from each of the two categories shown in table 1. Category 1 are monocotyledonous and category 2 are dicotyledonous.

6.3 Soil

Either sterile or non-sterile soil may be used. The field-moist soil shall be passed through a sieve, of square mesh 4 mm to 5 mm, to remove coarse fragments. The carbon content shall not exceed 1,5 % (3 % organic content). Fine particles (less than 0,02 mm) shall not exceed 20 % of dry mass. The pH determined in accordance with ISO 10390 shall be between 5 and 7,5. If the soil is prepared specifically for the purposes of the test and this involves the addition of nutrients, necessary precautions shall be taken to ensure that these nutrients do not interfere with the test substance (e.g. by allowing a sufficiently long period between preparation of the soil and running the test).

NOTE 1 It is recommended that sand should be added to bring the organic or fine particle content of natural soils to within the approved limits.

If non-sterile soil is used, it shall be stored in accordance with ISO 10381-6.

Table 1

Category	Test species
1	
Rye	*Secale cereale* L.
Ryegrass, perennial	*Lolium perenne* L.
Rice	*Oryza sativa* L.
Oat (common or winter)	*Avena sativa* L.
Wheat, soft	*Triticum aestivum* L.
Barley (spring or winter)	*Hordeum vulgare* L.
Sorghum, common (or shattercane or durra, white or millet, great)	*Sorghum bicolor* (L.) Moench
Sweetcorn	*Zea mays* L.
2	
Mustard, white	*Sinapis alba*
Rape [or rape (summer) or rape (winter)]	*Brassica napus* (L.) ssp. *napus*
Radish, wild	*Raphanus sativus* L.
Turnip, wild	*Brassica rapa* ssp. (DC.) Metzg.
Chinese cabbage	*Brassica campestris* L. var. *chinensis*
Birdsfoot fenugreek	*Trifolium ornithopodioides* (L.)
Lettuce	*Lactuca sativa* L.
Cress, garden	*Lepidium sativum* L.
Tomato	*Lycopersicon esculentum* Miller
Bean	*Phaseolus aureus* Roxb.

6.4 Soil treatment

Any method ensuring an even distribution of the chemical throughout the soil may be used, excluding the use of surfactants.

Recommended methods for incorporation of the chemical are described in annex A.

6.5 Recommended reference substance

NOTE 2 It is recommended that a reference substance be tested to demonstrate the uniformity of the laboratory test conditions. Sodium trichloroacetate is the recommended reference substance. A reference test should be carried out if any major changes in operating procedures are introduced, for example, change of phytotron/growth

room/greenhouse; change of soil or change of watering regime, etc.

7 Methods

7.1 Experimental design

Pots containing control soil and each concentration of chemical shall be replicated four times.

7.1.1 Preliminary test

The preliminary test is used to find the range of concentrations affecting soil quality. The chemical is incorporated in the soil according to annex A at concentrations of 0 (control), 1 mg/kg, 10 mg/kg, 100 mg/kg, 1 000 mg/kg, of oven-dried soil.

7.1.2 Final test

The concentrations shall be selected in a geometric series (preferably with a factor not exceeding two) to give an estimate of the lowest concentration that induces reduced emergence and growth (LOEC). Substances need not be tested at concentrations of higher than 1 000 mg/kg of oven-dried soil.

NOTE 3 A geometric series is a series of quantities in which each term is obtained by multiplying the preceeding term by some constant factor termed the common ratio, e.g. 1, 2, 4, 8, 16.

7.2 Preparation of the pots

Fill the pots with the prepared soil and obtain the required water holding capacity, expressed as a percentage, by adding deionized water. Place the pots on individual saucers and arrange them in a randomized block design.

7.3 Preparation of the seeds

Plant 20 uniform undressed seeds of the selected species from the same source either immediately after incorporation of the chemical or up to 24 h later. The seeds shall not be imbibed before planting.

7.4 Growing conditions

The temperature, humidity and light conditions shall be such that they are suitable for maintaining "normal" growth of all selected species for at least the duration of the test period. After the emergence assessment within each pot, thin the seedlings to give a total of five evenly spaced representative specimens of the plants in the pots. Terminate the test no sooner than 14 days and no later than 21 days after 50 % of the control seedlings have emerged.

NOTES

4 The following conditions and procedures are recommended

a) Testing facility: phytotron, plant growth room or greenhouse.

b) Temperature: to meet the normal growing conditions of the species selected.

c) Lighting: 16 h/day.

7 000 lx minimum light intensity in the wavelength suitable for photosynthesis. Therefore, in a greenhouse, additional lighting may be necessary during times of low natural light intensity.

d) Soil moisture content: daily adjustment of the moisture content of the soil is necessary to maintain a predetermined percentage water holding capacity e.g. 80 % for *Avena sativa* and 60 % for *Brassica rapa*. A sufficient check can be made by weighing several randomly selected pots daily. Anaerobic conditions should be avoided and noted in the test report.

e) Records: temperature and humidity, especially if using a greenhouse.

f) When testing volatile substances, interaction between batches should be avoided by using separate phytotrons or by specialized separation. If this is not possible, a note to this effect should be included in the test report.

5 A limit test may be performed under the conditions of this test in order to demonstrate that the LOEC is beyond the limit concentration.

7.5 Validity criteria

Emergence shall be sufficient to provide five healthy seedlings per pot in the control.

8 Assessment of results

8.1 Data presentation

Present the data in tabular form, recording the number of plants that emerge per replicate and the total mass of shoots of seedlings per replicate at harvest; either the fresh mass weighed immediately after cutting the shoots above the soil surface or the dry mass after oven drying at 70 °C to 80 °C for 16 h.

NOTE 6 To minimize the trial error it is preferable to use dry mass.