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Soil quality — Determination of the effects of pollutants on soil flora — Screening test for emergence of lettuce seedlings (*Lactuca sativa* L.)

*Qualité du sol — Détermination des effets des polluants sur la flore du sol — Essai de détection de l'émergence des plantules de laitue (*Lactuca sativa *L.)*

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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 17126 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

For the assessment of the suitability of soil to sustain living organisms, there is a need for simple, rapid, inexpensive biological test methods as a complement to chemical analysis. The method described in this International Standard has been developed for the testing of contaminated soil as well as other contaminated samples. It is cost effective and can be conducted within a short period of time. Furthermore, the test organism is easily available, it does not require advanced equipment for measurements or for growing plants, and it can be conducted by any skilled laboratory technician without special training.

This International Standard is based on US EPA 600/3-88-029 (1989)[1].

Soil quality — Determination of the effects of pollutants on soil flora — Screening test for emergence of lettuce seedlings (*Lactuca sativa* L.)

1 Scope

This International Standard specifies test procedures for the determination of effects of contaminated soils or other contaminated samples on the emergence of lettuce seeds.

This International Standard is applicable to contaminated soils, soil materials, compost, sludge and chemical testing. It is applicable to the measurement of effects of substances deliberately added to the soil and to the comparison of soils of known and unknown quality.

This International Standard is not applicable to volatile contaminants.

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 for the assessment of aerobic microbial processes in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 11265, *Soil quality — Determination of the specific electrical conductivity*

ISO 11267:1999, *Soil quality — Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants*

ISO 11274, *Soil quality — Determination of the water-retention characteristic – Laboratory methods*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

3 Terms and definitions

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

3.1
seedling emergence
appearance of the seedling (i.e. visible seedling) above the surface of the cover material

3.2
EC*x*
concentration of test material (or test substance) estimated to reduce the seedling emergence by x % as compared to the control

3.3
test material
material to be tested

EXAMPLES Soils, soil materials, compost, sludge.

3.4
test mixture
mixture of test material (or test substance) and growth medium

4 Principle

Lettuce seeds are exposed to the test material under investigation in a geometric dilution series with test material and growth medium. Incubation takes place under controlled conditions of light and temperature, and lasts usually 5 days (120 h). It is also possible to use this International Standard for chemical testing. In this case, seeds are planted in control pots and in pots containing soil to which the test chemical has been added. At the end of the test, the number of seedlings visible above the sand are counted and recorded. The effect on seedling emergence is expressed as EC50 (possibly EC20), calculated from numbers of emerged seedlings in the control pots (pure growth medium) and in pots containing the test material (or test substance).

5 Materials

5.1 Biological material, in this case lettuce seeds, *Lactuca sativa* L.

Seeds coated with insecticides and/or fungicides (“dressed” seeds) should be avoided.

After purchase, examine the seeds and remove any trash, empty hulls and damaged seeds. A uniform emergence is dependent on uniform seed size. To reduce variability of emergence, the seed batch may be sized before use by means of four sieves with oblong holes (see 6.4) placed on top of each other. In this case, select for testing the fraction with the largest number of seeds.

Pack the seeds in small portions in air-tight containers. The storage time of the seeds should not exceed the expiration date given by the supplier.

Storage at 4 °C is recommended but good emergence may also be accomplished by storage in the dark at 18 °C.

The seeds should not be soaked in water before testing.

5.2 Growth medium, in this case washed, fine quartz sand, e.g. with grain size 0,4 mm to 0,8 mm.

5.3 Cover material, in this case washed, coarse quartz sand, e.g. with grain size 0,7 mm to 1,2 mm (possibly 0,8 mm to 1,4 mm).

The coarse quality of the cover material ensures air exchange between the growth medium and the surroundings.

6 Apparatus

Standard laboratory equipment (pH-meter, thermometer, pipettes, etc.) including the following.

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

6.2 Lower parts of plastic Petri dishes (diameter 15 cm), or other containers with similar surface area, for use as test containers.

6.3 Re-sealable polyethylene bags that fit the test containers (20 cm × 25 cm for a 15 cm Petri dish).

6.4 Sieves for seeds, with oblong mesh dimensions of 0,75 mm × 10 mm, 0,8 mm × 10 mm, 0,85 mm × 10 mm and 0,9 mm × 10 mm.

6.5 Sieve for contaminated soil, stainless steel, with mesh size 2 mm.

6.6 Controlled environment chamber:

6.7 Magnifier

7 Procedure

7.1 Testing of samples of soil and other test materials

Usually the test material is not dried before the test. If necessary, upon reception air-dry it at room temperature to a water content that enables sieving. Immediately thereafter, sieve the test material through a stainless steel sieve (6.5) and store in the dark at 4 °C ± 2 °C until testing (in accordance with ISO 10381-6). Preferentially, storage should not exceed three months but if prolonged storage is necessary, it shall be stored at − 18 °C. For sieving, a mesh size of 2 mm is preferable but if this is not possible, coarser sieves (e.g. 5 mm) may be used.

Determine the following properties of the sieved test material and register them prior to the test:

— water content (in accordance with ISO 11465);

— water-holding capacity (in accordance with ISO 11274, or alternatively Annex C of ISO 11267:1999);

— conductivity (in accordance with ISO 11265);

— pH (in accordance with ISO 10390).

The water content and water-holding capacity of the test material and the water-holding capacity of the growth medium are used for calculation of the amounts of water to be used for the test. Before the test, weigh and mix the moist test material and the dry growth medium.

Use a geometric dilution series between test material and growth medium (minimum of 5 concentrations), for which the dilution factor should not exceed two. The range of concentrations should include those at which 0 (or minimum) and 100 % emergence are expected, e.g. based on a preliminary test (7.6.1).

The calculations are based on dry mass, and the concentrations are calculated and expressed as grams dry mass test material per gram dry mass test mixture (i.e. test material and growth medium).

NOTE A commonly used dilution factor is $\sqrt[4]{10}$ which is approximately 1,8, resulting in concentrations of e.g. 10, 18, 32, 56, 100.

7.2 Testing of chemicals

7.2.1 General

For testing of chemicals, the test is basically conducted with growth medium only and the chemical is added to the growth medium. The concentration of the chemical is calculated based on the dry mass of the growth medium using the data reported in Clause 8.

7.2.2 Water-soluble substances

Dissolve the chemical in water and add to the test dishes with the moisturizing water (see 7.4).

7.2.3 Substances insoluble in water

Dissolve the test substance in a volume as small as possible of a suitable organic solvent (e.g. acetone or ethanol). Then mix this solution (maximum 1 ml of solvent) with 10 g of the test growth medium per treatment and replicate. Allow the solvent to evaporate, add 90 g of growth medium and mix carefully in order to achieve a homogeneous distribution.

A set of control dishes should be prepared using the same amount of solvent.

7.3 Temperature and light regime

Incubate in a controlled environmental chamber at the optimum temperature that allows germination of the lettuce seeds. This may depend on the strain used (e.g. some strains germinate at 24 °C, while other seeds do not tolerate temperatures exceeding 20 °C). The temperature should be kept constant within ± 2 °C of the selected temperature.

During the first 48 h, store the test units in complete darkness. Thereafter, a diurnal cycle (16 h light, 8 h dark) should be maintained with fluorescent light at 4 300 lx ± 430 lx (30 $\mu E/m^2/s \pm 3\ \mu E/m^2/s$) for the remaining test period.

7.4 Water content

Moisten the test mixture with deionized water to approximately 85 % of the water-holding capacity. Retain the moisture in the experimental units during the test by using polyethylene bags. Measurement of water content during or after the test is thus not normally necessary.

7.5 Reference substance

It is recommended that a reference substance be tested to demonstrate the uniformity of the laboratory test conditions. 2-chloroacetamide or boric acid is suggested as reference substance. A reference test should be carried out regularly and after any major changes in operating procedures are introduced, for example change in phytotron/growth room/greenhouse, change in soil or change in watering regime, etc.

NOTE EC50 values for 2-chloroacetamide and boric acid are found to be 10,4 mg/kg and 406 mg/kg artificial soil, respectively. The value for 2-chloroacetamide is based on artificial soil consisting of silica sand, whereas the value for boric acid is based on artificial soil consisting of 70 % silica sand, 20 % kaolinite clay and 10 % sphagnum peat.

7.6 Preparation and start of test dishes

7.6.1 Preliminary test

In order to establish the range of concentrations of test material or chemical within which the effect is between 0 and 100 %, a preliminary test can be conducted.

For this, the procedures described for the final test (7.6.2) apply, with the exception that Petri dishes with a diameter of 9 cm with 15 seeds in each shall be used and only one replicate is necessary.

7.6.2 Final test

Weigh sufficient test material (moist) and growth medium (dry) to mix an amount equivalent to 300 g to 400 g dry mass of each dilution/concentration. Carefully mix test material and growth medium. Place an amount equivalent to 100 g dry mass of each test mixture in each of three replicate Petri dishes and smooth the surface.

For testing of chemicals, place 100 g dry mass of growth medium in each Petri dish.

As a control, prepare three dishes with growth medium only, following the same procedure as for the test dishes.

Place 40 lettuce seeds on top of the test mixture/growth medium. Distribute the seeds evenly over the area but not closer than 1 cm from the edge of the test container. Press the seeds gently into the medium, e.g. using the bottom of a clean beaker.

Moisten the contents of the dishes with the amount of water calculated to obtain 85 % of the water-holding capacity. Spread the water evenly over the surface.

For chemical testing of water-soluble substances, dissolve the substance in the water before the growth medium is moistened. If soil characteristics cause floating of the seeds upon addition of water, the seeds should be placed in the test container after addition of water.

Cover the contents of each Petri dish evenly with 90 g of dry cover material.

Between operations, cover the dishes with the lid in order to reduce evaporation. Immediately before the dishes are placed in polyethylene bags, remove the lid. Elevate the polyethylene bag to allow room for air in each bag before sealing the bags.

Randomly place the Petri dishes in bags inside the controlled environment chamber for incubation. The specified light regime (7.3) should be ensured during incubation.

7.7 Test duration

Continue incubation until emergence of seedlings has been completed in control dishes, usually 120 h. Depending on the temperature used for the emergence (see 7.3), this period may have to be adjusted. However, the test duration shall not exceed 7 days.

7.8 Measurements

At the beginning of the test period and at the end of the test period (usually 120 h), measure and record the pH and conductivity of samples from moistened sand (control) and from the test mixture least diluted with growth medium. Make both measurements on the overall medium, i.e. test mixture plus cover material.

Temperature shall be recorded daily in the chamber and inside one of the bags, chosen at random, in order to ensure that the temperature is not elevated inside the bags.

7.9 Recordings

At the end of the test, determine the number of emerged seedlings by counting each seedling that has emerged (i.e. is visible) above the surface of the cover material. Additional information may be obtained by careful inspection of the seedlings. Any observed effects should be recorded.

8 Expression of results

The results, i.e. the number of seedlings emerged, are expressed by means of probit analysis or other applicable statistical methods, as the EC50, which corresponds to 50 % of the mean seedling emergence in the controls, or the EC20, which corresponds to 80 % of the mean seedling emergence in the controls. EC50 and/or EC20 shall be expressed as grams dry mass test material per gram dry mass test mixture.