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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

Reference number
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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 $\pm 2s$
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 $\times$ 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.

For limit tests, it is recommended that the number of replicates be increased in order to improve the power of the statistical analysis.

9.2 Preparation of pots

Depending on the nature of the test material, the test mixtures are prepared in accordance with Annex D (chemicals) or Annex E (compost, sludge, waste).

The test vessels (6.1) are filled with the sand, the test soil, the control soil or the test mixtures to approximately 5 mm to 10 mm below the upper edge. All pots of each treatment should contain the same volume of soil. The soil should not be compressed strongly.

The sand, test soil, control soil or test mixtures shall be wetted with deionized water to reach 70 % ± 5 % of the total water-holding capacity determined in accordance with Annex A.

The pH (KCl) for each treatment (one vessel per treatment) is measured in accordance with ISO 10930.

9.3 Pregermination of the seeds

Germinate the seeds in a Petri dish, evenly distributed on a bed of filter paper moistened with deionized water, until the radicle has just emerged:

— for barley, normally 36 h to 48 h at 20 °C in the absence of light;

— for oat, normally 48 h to 72 h at 20 °C in the absence of light;

— for wheat, normally 48 h to 60 h at 20 °C in the absence of light.

NOTE The time intervals given above are only indicative. They were established using a limited number of varieties per species. The optimal duration of germination can be influenced both by the variety and density of seeds in the Petri dish.

When the radicle has emerged but is less than 2 mm in length, plant in each pot six seeds, radicle down, approximately 10 mm beneath the surface of the test medium.

9.4 Growing conditions

Incubate the pots in a controlled environmental chamber (7.1).

Table 3 — Conditions recommended for monocotyledonous species

Condition	Day	Night
Photoperiod	12 h to 16 h	8 h to 12 h
Light intensity	Min. 2 500 lx	—
Temperature (°C)	20 ± 2	16 ± 2
Moisture content (% C_W)	70 ± 5	70 ± 5

A constant moisture content shall be achieved during the exposure period. It has been proven that placing vessels in polyethylene bags (7.3) allows this requirement to be fulfilled without any additional watering.

In order to prevent any effects of unequal lighting and temperature on the growth of the test plants, the test vessels are disposed randomly in the controlled environmental chamber.

NOTE For clayey soils, it might be necessary to reduce the soil moisture content.

9.5 Test duration

Incubate the pots for four days in the conditions defined in 9.4.

NOTE For dicotyledonous species, it might be necessary to adapt the growth time.

9.6 Measurements

After the growth period, lay each pot on its side and press it manually with care to remove the soil from the pot. Separate the plants from the soil (see Annex F). Wash each plant and measure the longest root to the nearest 0,5 mm.

The root length should be measured from the transition point between the hypocotyl and root to the tip of the root.

The shoot height may also be measured in conjunction with the root length to provide additional or corroborative data.

NOTE Plants can be easily separated from compact soils by laying the pot in a tray to which water is added (layer about 5 cm thick).

At the end of the test, measure the pH (KCl) for each treatment (one vessel per treatment) in accordance with ISO 10390.

10 Expression of results and data

Measure the length of the longest root of each plant and determine the mean length on the longest root for each test condition (sand control, control soil, test mixtures or undiluted test soil). Present the data in tabular form.

Compare the mean root lengths of the test mixtures or undiluted test soil to those of the control vessels. First, a statistical analysis of the homogeneity of the variances shall be made. With homogeneous data, an appropriate statistical analysis (e.g. one-way analysis of variance (ANOVA) followed by a Dunnett test ($\alpha = 0{,}05$) should be performed. If the homogeneity requirement is not fulfilled, it is recommended to evaluate whether an appropriate transformation of the data could solve the problem. Otherwise, non-parametric methods can be used.

If a limit test has been performed and the prerequisites (normality, homogeneity) of the parametric method are fulfilled, the Student-t-test or the Mann-Whitney-U-test procedure should be used.

When a full test has been performed, EC_x (10, 20, 50) can be calculated if a clear dose–response relationship is found. Use any suitable statistical procedures to calculate the EC_x (10, 20, 50) with confidence limits (P = 0,95). See ISO/TS 20281 for information.

11 Validity criteria

The results are considered to be valid if:

— the root elongation of the selected species exhibits normal growth in the sand control, reaching a length within the range given in 6.2.3;

— the mean length on the longest root shall be within the $\pm 2s$ range of the mean obtained with the selected strain;

— the coefficient of variation of root elongation in the sand control does not exceed 20 %.

12 Test report

The test report shall include the following information:

a) a reference to this part of ISO 11269;

b) a full description of the experimental design and procedures;

c) the test plant species (variety, source);

d) the test environment (temperature, photoperiod, lighting, etc.);

e) the characteristics of the test soil (if appropriate);

f) the characteristics of the test material: compost, sludge, waste (if appropriate);

g) the characteristics of the sand;

h) the characteristics of the control soil;

i) the length of the longest root of each plant within the pots containing

   1) sand,

   2) control soil,

   3) test sample,

   4) test mixtures;

j) the shoot height (if measured);

k) any other effects observed;

l) the results of the test (in the form of a table) including treatment, replicate number and length of the longest root on each plant. Indicate whether growth inhibition is statistically significant or the level of significance of any growth inhibition observed.

Annex A
(informative)

Recommended method for measuring the water-holding capacity of the soil

Fill a tube of known volume with the soil, with the base closed by a sheet of filter paper, and cap the tube. Submerge the tube in a water bath at room temperature (with the water level beneath the top of the tube) for 2 h. Then lower the tube below the water level for a further 1 h. Place the tube in a tray of wet, finely ground, quartz sand to drain for 2 h. Weigh the sample and dry it to constant mass at 105 °C.

The water-holding capacity, C_w, is calculated as a percentage of dry mass, with the help of the following equation:

$$C_w = \frac{m_s - m_t - m_d}{m_d} \times 100 \quad \text{(A.1)}$$

where

m_s is the water-saturated soil mass, plus the tube mass and the filter paper mass;

m_t is the tare (the tube mass and the filter paper mass);

m_d is the dry mass of the soil (the mass of the tube with dry soil and filter paper less the tare mass of the tube and filter paper).

Annex B
(informative)

Results of tests performed on reference substances

B.1 Nickel sulfate ($NiSO_4 \cdot 6H_2O$)

Species	Substrate	Duration	EC_{50} values mg/kg, expressed as Ni^{2+}
Winter barley	Sand	4 days	1,01 (0,70 – 1,45) 2,32 (1,87 – 2,88)
		5 days	2,34 (1,01 – 5,42) 0,81 (0,60 – 1,10) 1,65 (1,12 – 2,45) 1,21 (1,05 – 1,40) 2,17 (1,97 – 2,44) 1,50 (1,04 – 2,16) 1,33 (1,21 – 1,47)
Oat	Sand	4 days	1,86 (1,43 – 2,43)

B.2 Boric acid (H_3BO_3)

Species	Substrate	Duration	EC_{50} values mg/kg
Winter barley	Sand	4 days	62,3 (55,8 – 69,9) 70,3 (63,2 – 78,7) 61,9 (54,9 – 70,2)
Oat	Artificial soil	4 days	449,2 (405,7 – 505,3) 255,0 (148,1 – 440,3)
		5 days	365,4 (322,8 – 412,2)
Wheat	Sand	4 days	57,5 (50,5 – 65,5) 63,0 (44,0 – 90,5) 57,8 (48,8 – 67,1)
	Artificial soil	4 days	281,5 (166,0 – 479) 294,0 (216,7 – 399,6)
		5 days	278,1 (211,7 – 365,3) 487,5 (438,4 – 552,4)

Annex C
(informative)

Example of results obtained with boric acid using sand as the substrate

Table C.1 — Length of the longest roots measured in all replicates of each test condition

Concentration of boric acid mg/kg	Root length (winter barley) mm						Mean length mm
Control	134	126	135	125	127	137	
	122	137	135	135	140	136	
	140	138	141	165	131	145	
							136,1
10,0	146	130	131	130	127	125	
	137	137	116	111	131	132	
	131	122	137	119	137	122	
							128,9
18,0	122	117	116	109	131	140	
	105	135	115	117	122	118	
	125	100	101	119	127	120	
							118,9
32,4	110	112	109	109	115	107	
	116	94	110	104	104	107	
	102	96	104	101	115	109	
							106,9
58,3	76	72	65	69	74	79	
	81	78	80	75	78	76	
	79	67	83	76	82	75	
							75,8
105,0	44	39	38	40	37	37	
	38	36	36	42	35	35	
	37	35	35	34	39	36	
							37,4
189,0	17	17	17	17	16	15	
	16	16	15	15	15	16	
	17	16	19	16	19	15	
							16,3

Based on these results, the EC_{50} (4 days) calculated is 61,9 mg/kg (54,9 – 70,2).

Annex D
(informative)

Recommended methods for the incorporation of chemicals into soils

D.1 Water-soluble substances

Dissolve the quantity of test substance required to obtain the desired concentration in deionized water (water used to wet the soil). Mix it thoroughly with the soil (reference or standard) partly moistened.

The test mixture shall be wetted with deionized water to reach 70 % ± 5 % of the total water-holding capacity determined in accordance with Annex A.

D.2 Substances insoluble in water but soluble in organic solvents

Dissolve in a volatile solvent (such as methanol or acetone) the quantity of test substance required to obtain the desired concentration and mix it thoroughly with a portion (10 g to 50 g) of the quartz sand (see 6.2). Evaporate the solvent by placing the container under a fume hood.

After evaporation of the solvent, the sand is mixed thoroughly with the soil and the deionized water to reach 70 % ± 5 % of the total water-holding capacity.

D.3 Substances insoluble in water and organic solvents

For a substance that is insoluble in a volatile solvent, prepare a mixture of industrial quartz sand (10 g to 50 g) and the quantity of the test substance required to obtain the desired concentration. Transfer this mixture to a glass container filled with the soil and mix thoroughly.

Add deionized water to reach 70 % ± 5 % of the total water-holding capacity of the soil, and mix thoroughly.

Annex E
(informative)

Recommended methods for the incorporation of compost, sludge or waste into soils

E.1 General

The preparation of test mixtures differs according to the material to be tested. The different methods of preparation are described below. The material, originally or after pretreatment, shall have a particle size less than 2 mm.

Depending on the purpose of the test, sand can be used as an alternative to soil for assessing the effects of compost, sludge or waste. This can be particularly relevant when comparing ecotoxicological properties of different test materials (e.g. for regulatory purposes).

On the other hand, the use of sand is not recommended if the experiment aims to assess the effects of adding sludge or compost to land when the area of application is known.

E.2 Solid materials

Different methods can be applied to introduce the test material into the soil. Several parameters influence the selection of the introduction method, such as physical properties or amounts to be tested. The following methods are recommended:

— for small amounts, introduce the test material into the water (or into part of the water) necessary to wet the soil, then mix this suspension thoroughly with the soil;

— for large amounts, mix the test material thoroughly with the already hydrated soil;

— for hydrophobic material, mix the test material thoroughly with the soil, then add the water necessary to wet the mixture.

E.3 Liquid sludge or liquid waste miscible with water

Introduce the test material into the water (or into part of the water) necessary to wet the soil, then mix this suspension thoroughly with the soil.

The volume of liquid sludge or liquid waste to be added is limited to 70 % $\pm$ 5 % of the total water-holding capacity of the test mixture.

E.4 Liquid waste non-miscible with water

The following methods are recommended.

— For small amounts:

  introduce, by ultrasonic dispersion, the test material into the water (or into part of the water) necessary to wet the soil, then mix this suspension thoroughly with the dilution medium; or

  prepare a mixture of quartz sand and the quantity of test material required to obtain the desired amount (a ratio of 10 g of sand per kilogram of soil is usually recommended); mix with the soil thoroughly, then add the water necessary to wet this mixture.

— For large amounts:

  mix the test material thoroughly with the soil already hydrated; or

  mix the test portion thoroughly with the soil, then add the water necessary to wet this mixture.

Annex F
(informative)

Example of seedlings of winter barley collected at the end of the test after removal from artificial soil

Key

1 sand control

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