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भारतीय मानक

मृदा पादकों पर पर्यावरण का प्रभाव ज्ञात करना

भाग 1 जड़ों की वृद्धि में रुकावट मापने की विधि

Indian Standard

DETERMINATION OF THE EFFECTS OF POLLUTANTS ON SOIL FLORA

PART 1 METHOD FOR THE MEASUREMENT OF INHIBITION OF ROOT GROWTH

ICS 13.080

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Price Group 5

NATIONAL FOREWORD

This Indian Standard (Part 1) which is identical with ISO 11269-1 : 1993 'Soil quality — Determination of the effects of pollutants on soil flora — Part 1 : Method for the measurement of inhibition of root growth' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Soil Quality and Improvement Sectional Committee and approval of the Food and Agriculture Division Council.

In the adopted standard, certain terminology and conventions are not identical to those used in Indian Standards. Attention is particularly drawn to the following:

a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.

b) Comma (,) has been used as a decimal marker while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

CROSS REFERENCES

International Standard	*Corresponding Indian Standard*	*Degree of Equivalence*
ISO 11274 : 2001 Soil quality — Determination of the water retention characteristic — Laboratory methods	Doc : FAD 27 (1318) 1 Soil quality — Determination of the water retention characteristic — Laboratory method	Identical

In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

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 preliminary test for the rapid estimation of soil quality by comparing the rate of growth of roots of a specified plant under standard conditions with that in a test soil.

The method is applicable to all soils, soil forming materials, waste residues or chemicals which may be applied to soil, except where the contaminant is highly volatile or only affects photosynthesis.

It is applicable to the measurement of the effects of substances deliberately added to the soil and to the comparison of soils of known and unknown quality.

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

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 11269. At the time of publication, the edition indicated was 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 edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 11274:—[1], *Soil quality — Determination of the water retention characteristic — Laboratory methods.*

1) To be published.

3 Principle

Growth of pregerminated seeds under controlled conditions for a set period depending on the test plant being used. The two control media are sand and soil. After the growth period, measurement of the root lengths from both of the controls and the unknown soil or substance under test. Statistically significant differences in the root lengths of seedlings grown in any test medium compared to the controls is indicative of an effect.

NOTE 1 Shoot length is also a useful parameter, and this can be measured in conjunction with root length to provide additional or corroborative data.

4 Test plants and materials

4.1 Plants

The test plant shall be grown from undressed seeds.

NOTES

2 Barley (*Hordeum vulgare* L.) of the variety "CV Triumph" has been used for several years and is currently recommended. However, other barley varieties (undressed) will suffice, providing they have similar qualities of germination and root elongation.

3 the methodology of this test can also be adapted for use with dicotyledenous species with straight roots which are easily measurable.

4.2 Pots

The pots shall be cylindrical, approximately 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 if necessary.

NOTE 4 When filled to a height of 10 cm, the pots will contain approximately 500 g of air-dry soil.

4.3 Growth media

The growth media are the test soil, a control soil that is known to be of good quality and a sand control.

The sand control medium is 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 5 The control soil and test soil 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.

5 Methods

5.1 Experimental design

Growth in three growth media (4.3), a sand control, a known good quality soil preferably of the same textural class as the soil under test and the unknown soil, are each replicated three times. The sand control is used to confirm the reproducibility of the test carried out on different occasions, whilst the extents of growth in two soils are compared statistically.

The method may also be used to determine the possible toxic effects of solid or liquid chemicals incorporated into the soil (see annex A).

NOTE 6 If the test is conducted to determine the effect of added substances, and the type of soil used is not an essential requisite, the soil should be chosen so that it does not mask or reduce the effect of added substances.

5.2 Preparation of pots (4.2)

5.2.1 General

Dry the industrial sand, test soil or soil to which test substances will be added at (30 ± 2) °C for 16 h, and then pass it through a 4 mm sieve. Prepare material for the control pots (5.2.2) and either soil containing the test substance (5.2.3) or unknown soil (5.2.4), as appropriate.

5.2.2 Control pots

Fill three weighed pots with industrial sand, ensuring that the material is not compacted in any way.

5.2.3 Soil containing test substances (if appropriate)

Dry and sieve the control soil into weighed pots, taking care to avoid compaction. Prepare sufficient pots for the three replicates of the soil control and all treatments. Select the inclusion rates for a preliminary or final test according to annex A. Incorporate the test substance using one of the methods described in annex B.

5.2.4 Unknown soil pots (if appropriate)

Dry and sieve the unknown soil. Fill three weighed pots, taking care to avoid compaction. Fill additional sets of three pots with mixtures of the unknown soil and either control soil which has been dried and sieved or industrial sand, to prepare diluted samples containing various concentrations of substances in the unknown soil.

5.3 Preparation for sowing

During this test, ensure that the soil is at 70 % water holding capacity (whc) by either of the following two methods:

a) Stand the three pots of each soil type in a close fitting trough with a water depth maintained between 5 cm and 10 cm.

   When the surface of the soil is wet, remove the pot, cover it with a watch glass and allow it to stand on a rack overnight to drain. The soil is then considered to be at approximately 100 % whc capacity. Reweigh the pots and allow to dry by evaporation to 70 % whc and maintain at this mass for the entire test period.

b) Determine the mass of water required for 70 % whc in accordance with ISO 11274 on a separate soil sample. Add that mass of water to the pots (via either the top or the bottom), taking care to minimize the compaction of the soil surface, and maintain at this mass for the entire test period.

NOTES

7 It is preferable to use deionized water.

8 Method b) is particularly useful when studying water-soluble chemicals.

5.4 Preparation of the seeds

Germinate the seeds in a Petri dish, evenly distributed on a bed of filter paper moistened with distilled water, until the radicle has just emerged, e.g. normally 36 h

to 48 h for barley at 20 °C in the absence of light. When the radicle has emerged but is less than 2 mm in length, plant six seeds, radicle down, approximately 10 mm beneath the surface of the test medium.

5.5 Growing conditions

Place the pots in a growth cabinet with preset day and night conditions. Weigh the pots daily and add deionized water to maintain the soil at 70 % whc, taking care to avoid compaction, and replace them in a randomized design.

NOTE 9 The following conditions are recommended and have been found suitable for barley.

Condition	Day	Night
Duration (h)	12 to 16	8 to 12
Lighting	25 000 lm/m^2	Tungsten 45 W
Temperature (°C)	20 ± 2	16 ± 2
Humidity (%)	60 ± 5	60 ± 5
Moisture (% whc)	70 ± 5	70 ± 5

If barley is used as the test plant (4.1), use a growth time of 5 d. If barley is not the test plant, first grow the chosen test plant in sand to determine the maximum root length obtainable from seed reserves. Ensure that the root length does not exceed 80 % of the depth of the soil in the test pots by a suitable choice of pots and growth time. Select a growth time that is known to produce roots no longer than 80 % of the depth of the soil in the pot.

NOTE 10 If the method is being used to investigate the growth of plants other than barley, growth times longer or shorter than 5 d may be necessary.

After the appropriate growth period, lay each pot on its side in a trough of water 5 cm deep, and wash the soil gently out of the pots. Wash each plant and measure the longest root to the nearest 0,5 mm.

NOTE 11 The shoot can also be measured if desired.

6 Expression of results (see annex D)

6.1 Data

Measure the length of the longest root of each plant and determine the mean length of the longest root for each growth medium or treatment level tested. Compare the mean root lengths of the treatments to those from the control pots. Evaluate the results using a suitable statistical test.

NOTE 12 Student's *t*-test or Dunnett's *t*-test are examples of suitable methods (see annex C).

A soil of poor quality induces a significant reduction in root length compared to good soil or the control sand.

6.2 Precision

In an interlaboratory test involving six laboratories, all were able to determine at the 95 % confidence level that a 10 mm difference in mean root length (approximately 10 %) was significant. (See annex E.)

7 Test report

The test report shall include the following information:

a) a reference to this part of ISO 11269;

b) a method of uniquely identifying the soil and its source;

c) variety of seed, or details of other plant species used;

d) growing conditions;

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

   1) sand,

   2) soil,

   3) test sample;

f) a description of the visual appearance of the soil and site, if available;

g) any other effects observed;

h) the results of the test (in the form of a table) including treatment, replicate number and length of the longest root on each plant, and whether or not any growth inhibition is statistically significant or the level of significance of any growth inhibition observed.

Annex A
(informative)

Adaptation of the method for added substances

A.1 Preliminary test

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

A.2 Final test

Select the concentrations in a geometric series with a factor preferably not exceeding 2, to give an estimate of the lowest concentration that induces growth reduction (LOEC). Substances need not be tested at concentrations greater than 1 000 mg/kg of oven-dried soil.

Replicate pots containing control soil and each concentration of chemical three times.

NOTE 13 A geometric series is a series of quantities in which each term is obtained by multiplying the preceding term by a constant factor termed the common ratio, e.g. 1, 3, 9, 27, 81 ... .

Annex B
(informative)

Recommended methods for incorporation of chemicals into soils

B.1 Chemicals soluble in water

Dissolve the chemical in water and mix directly with the soil. Ensure that the same quantity of water is used for each batch of soil and for each concentration of chemical.

B.2 Chemicals with low water solubility

Dissolve the chemical in water and mix with sand. A rotating drum is useful for this. Mix the treated sand with soil. If large quantities of water are required, the sand can be dried in the rotating drum, with a current of air, before mixing with the soil.

B.3 Chemicals soluble in a solvent

Dissolve the chemical in a suitable volatile solvent and mix with sand. Dry the sand in a stream of air while continuing mixing (e.g. while rotating the drum as above). Mix the treated sand with soil. Ensure that the same quantity of solvent and the same quantity of sand are used for all treatments, including the control.

Annex C
(informative)

Examples of statistical treatment of results

C.1 Student's *t*-test

Root lengths measured

Pot A: 27,6; 23,8; 22,9; 28,2; 22,5 Mean 25,0

Pot B: 28,3; 33,5; 27,2; 31,1; 29,9 Mean 30,0

To attach confidence limits to the means, tabulated values of t at four degrees of freedom are used (i.e. 2,776).

$$\text{Upper limit} = \text{Mean} + t \times \frac{s}{\sqrt{n}}$$

$$= 28,3 \text{ (for pot A)}$$

$$= 32,3 \text{ (for pot B)}$$

$$\text{Lower limit} = \text{Mean} - t \times \frac{s}{\sqrt{n}}$$

$$= 21,7 \text{ (for pot A)}$$

$$= 27,7 \text{ (for pot B)}$$

where

s is the standard deviation;

n is the number of measurements.

To assess significant differences between the pots, use the equation

$$t = \frac{\bar{x}_A - \bar{x}_B}{\frac{s_A^2}{n_A} + \frac{s_B^2}{n_B}}$$

$$= 3,215$$

In this example, the value of t exceeds the tabulated value of t at 0,02 with eight degrees of freedom. Therefore, the samples are significantly different at the 98 % confidence level.

C.2 Dunnett's *t*-test[2)]

Root lengths measured

Pot A: 27,6; 23,8; 22,9; 28,2; 22,5 Mean 25,0

Pot B: 25,2; 29,9; 26,4; 28,7; 27,3 Mean 27,5

Pot C: 28,3; 33,5; 27,2; 31,1; 29,9 Mean 30,0

Degrees of freedom (ν)

ν = Treatments × (Measurements −1)

$= 3 \times (5 - 1) = 12$

$$S^2 = \frac{\sum \bar{x}_i^2 - n \sum \bar{x}_j^2}{\nu}$$

$$= \frac{11\,473,29 - 11\,406,25}{12} = \frac{67,04}{12} = 5,58$$

$$S = \sqrt{5,58} = 2,362$$

where

$\sum \bar{x}_i^2$ is the sum of measurements squared;

$n \sum \bar{x}_j^2$ is the number of measurements in each pot × Sum of squares of the mean.

Standard error (SE)

$$\text{SE} = S \times \sqrt{\frac{T-1}{N}}$$

$$= 2,362 \times \sqrt{\frac{2}{5}}$$

$$= 1,494$$

where

T is the number of treatments;

N is the number of measurements per treatment.

Significant difference = t × SE

Where t (taken from Dunnett's table 2[2)]) = 2,50

Significant difference = 2,5 × 1,494 = 3,735

Pot A is therefore significantly different from Pot C (the control) at the 95 % confidence level. However pot B is not significantly different from the control.

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2) Dunnett, C.W. Multiple comparison procedure for several treatments, *Journal of American Statistical Association*, Vol. **50** (1955), pp. 1096-1121.

Annex D
(informative)

Example of test results

Nickel concentration mg/kg	Root lengths mm						Mean length mm
0	98	99	105	100	100	101	101
	102	103	97	101	104	102	
	97	100	99	96	102	105	
50	96	104	99	101	102	97	99
	102	100	97	98	98	100	
	99	97	96	100	103	98	
100	85	91	93	92	88	84	88
	90	84	89	90	87	93	
	88	87	85	86	82	84	
500	17	9	8	11	16	13	12
	11	12	9	15	15	7	
	8	15	17	10	12	9	
1 000	9	3	4	11	3	7	6
	7	5	8	7	4	5	
	5	6	9	7	6	8	

Annex E
(informative)

Results of interlaboratory test of root elongation

The methodology for the test to validate the proposed standard method was circulated to all standards organizations of participating member countries or the member of the working group representing a member country.

Results were obtained from only three countries: UK, Germany and France who completed four, three and one test(s) respectively. Not all eight of the laboratories conducted the test correctly or supplied sufficient raw data. However six laboratories completed all aspects of the test as envisaged.

Table E.1 — Statistical data

Standard errors	Significant differences mm
16,5	8,3
18,3	9,8
6,4	37,5[1)]
3,1	2,9
7,2	6,7
6,3	5,9
10,3	9,6
10,8	8,7

1) No raw data; only means were provided.

The results of root lengths for each set of results have been tabulated in a standard format from which means, standard errors and significant differences according to Dunnett's test have been calculated.

Standard errors, calculated as shown in C.2 and significant differences, expressed as root length in millimetres, have been tabulated for each set of results in table E.1.

The significant differences in themselves are only an indication of the accuracy of the test, but when compared to the main root length of the control the significant difference usually equates to 10 % of the root length. Thus, any chemical in the soil producing a 15 % reduction in root length almost certainly has a significant effect on root growth. Therefore, this simple root test can be used to rapidly assess the presence of factors in the soil which inhibit maximum crop growth.

Annex F
(informative)

Protocol for ring test

This protocol is designed to establish the precision of the root elongation test when carried out internationally.

a) This part of ISO 11269 shall be followed.

b) Use the barley seeds provided.

c) The only test substrate is sand (as specified in this part of ISO 11269).

d) The water holding capacity shall be determined initially on a separate sample of sand. From this, the quantity of water required for each pot is calculated.

e) Prepare solutions of analytical grade nickel sulfate containing 50 ppm, 100 ppm, 500 ppm and 1 000 ppm of Ni.

f) Prepare sample pots of sand containing a water control and four levels of nickel sulfate, by adding a sufficient volume of either water or one of the four solutions with different levels of nickel sulfate to each of three pots to attain 70 % whc.

g) Continue as described in 5.4 of this part of ISO 11269. Maintain 70 % whc with deionized water in all cases.

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Review of Indian Standards

Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards : Monthly Additions'.

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