SVENSK STANDARD
SS-EN ISO 11269-2:2013

Fastställd/Approved: 2013-02-25
Publicerad/Published: 2013-02-27
Utgåva/Edition: 1
Språk/Language: engelska/English
ICS: 13.080.30

SWEDISH STANDARDS INSTITUTE

Markundersökningar – Bestämning av föroreningars effekt på markfloran –
Del 2: Effekter av kontaminerade jordar på högre växters grobarhet och tidig tillväxt (ISO 11269-2:2012)

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 (ISO 11269-2:2012)

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The European Standard EN ISO 11269-2:2013 has the status of a Swedish Standard. This document contains the official version of EN ISO 11269-2:2013.

Förhållandet till övriga delar under samma huvudtitel - Utdrag ur Förord i ISO 11269-2:2012/ Relations to other parts under the same general title - Extract from the Foreword of ISO 11269-2:2012

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

Denna standard är framtagen av kommittén för Karaktärisering av avfall, mark och slam, SIS/TK 535.

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 11269-2

February 2013

ICS 13.080.30

English Version

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 (ISO 11269-2:2012)

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 (ISO 11269-2:2012)

Bodenbeschaffenheit - Bestimmung der Wirkungen von Schadstoffen auf die Bodenflora - Teil 2: Wirkung von verunreinigten Böden auf Saatauflauf und frühes Wachstum höherer Pflanzen (ISO 11269-2:2012)

This European Standard was approved by CEN on 5 February 2013.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN ISO 11269-2:2013: E

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Foreword

The text of ISO 11269-2:2012 has been prepared by Technical Committee ISO/TC 190 “Soil quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 11269-2:2013 by Technical Committee CEN/TC 345 “Characterization of soils” the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2013, and conflicting national standards shall be withdrawn at the latest by August 2013.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 11269-2:2012 has been approved by CEN as EN ISO 11269-2:2013 without any modification.

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 .

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.

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 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.

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 ($\rho < 0{,}05$)

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

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

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.