SLOVENSKI STANDARD
SIST ISO 22030:2006

01-december-2006

Kakovost tal ï Biološke metode ï Kroniļ na toksiļ nost za viģje rastline

Soil quality -- Biological methods -- Chronic toxicity in higher plants

Qualité du sol -- Méthodes biologiques -- Toxicité chronique sur les plantes supérieures

Ta slovenski standard je istoveten z: ISO 22030:2005

ICS:

13.080.30 Biološke lastnosti tal Biological properties of soils

SIST ISO 22030:2006 **en**

INTERNATIONAL STANDARD

ISO 22030

First edition
2005-02-01

Soil quality — Biological methods — Chronic toxicity in higher plants

Qualité du sol — Méthodes biologiques — Toxicité chronique sur les plantes supérieures

Reference number
ISO 22030:2005(E)

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Published in Switzerland

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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 22030 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

This International Standard describes a procedure for evaluating the quality of soils of different origin carrying unknown contaminations. The method, slightly modified, can also be used to measure the toxicity of known chemicals incorporated into soil.

The evaluation of the inhibition and chronic toxicity is based on emergence, vegetative growth and reproductive capacity of at least two species of higher plants.

This International Standard is based on:

a) results of the research project “Development of a chronic bioassay using higher plants”, sponsored by the German Ministry for Education and Research (BMBF), Bonn [3], and

b) discussions within the joint project “Ecotoxicological Test Batteries” forming part of the BMBF Joint Research Group “Processes for the Bioremediation of Soil” [10].

Soil quality — Biological methods — Chronic toxicity in higher plants

WARNING — Contaminated soils can contain unknown mixtures of toxic, mutagenic or otherwise harmful chemicals or infectious microorganisms. Occupational health risks can arise from dust or evaporated chemicals during handling and incubation. Furthermore, test plants can absorb chemicals from the soil and safety measures should also be considered when handling these test plants.

1 Scope

This International Standard describes a method for determining the inhibition of the growth and reproductive capability of higher plants by soils under controlled conditions. Two species are recommended: a rapid-cycling variant of turnip rape (*Brassica rapa* CrGC syn. Rbr) and oat (*Avena sativa*). The duration of test should be sufficient to include chronic endpoints that demonstrate the reproductive capability of the test plants.

By using natural test soils, e.g. from contaminated sites or remediated soils, and by comparing the development of the test plants in these soils with reference or standard control soils, the test can be used to assess soil quality, especially the function of the soil as a habitat for plants.

Annex A describes modifications allowing use of the chronic plant assay for the testing of chemicals incorporated into soil. By preparing a dilution series of a test substance in standard control soils, the same endpoints can be measured to assess the chronic toxicity of chemicals. This method is not applicable to volatile substances, i.e. substances for which *H* (Henry's constant) or the air/water partition coefficient is greater than 1, or for which the vapour pressure exceeds 0,013 3 Pa at 25 °C.

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 11268-1:1993, *Soil quality — Effects of pollutants on earthworms (Eisenia fetida) — Part 1: Determination of acute toxicity using artificial soil substrate*

ISO 11268-2:1998, *Soil quality — Effects of pollutants on earthworms (Eisenia fetida) — Part 2: Determination of effects on reproduction*

ISO 11269-2, *Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of chemicals on the emergence and growth of higher plants*

ISO 15176:2002, *Soil quality — Characterization of excavated soil and other soil materials intended for re-use*

ISO 15799, *Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials*

ASTM D1076:2002, *Standard Specification for Rubber-Concentrated, Ammonia Preserved, Creamed, and Centrifuged Natural Latex*

3 Terms and definitions

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

3.1
artificial soil
mixture of sand, kaolinite, peat and calcium carbonate

NOTE ISO 11268-1 describes such a soil for toxicity tests using earthworms. Pure quartz sand, mineral wool, vermiculite or other synthetic substrates should not be used.

3.2
biomass
total mass of shoots, flowers and seed pods

NOTE 1 Biomass is expressed as dry mass per plant or, if needed, as dry mass per pot.

NOTE 2 During the test period, some of the test plants can reach different growth stages and their water content can differ when the plants are harvested. Thus the dry mass better represents the biomass produced during the growth period.

3.3
concentration
mass of test substance per amount of soil

NOTE Concentration is expressed as a mass fraction, in milligrams per kilogram (mg/kg) of dry soil.

3.4
contaminant
substance or agent present in the soil as a result of human activity

[ISO 15176:2002]

3.5
control soil
uncontaminated substrate, used as a control and as medium for preparing dilution series with test soils or chemicals, that allows the growth of healthy plants

NOTE Either artificial or natural standard or reference soils can be used, if unhindered growth of the test plants in these soils can be expected. In any case, differences in nutrient levels between a test soil and a control soil can affect the dose-response pattern. For example, a control soil much richer in nutrients than a test soil can result in a false positive result (i.e. the test soil appears to have a “toxic” effect on the growth of the test plants). If a control soil is poorer in nutrients than a test soil, hormesis (see 3.9) can be expected at low soil-mixture ratios, or even an inverse dose response relationship, if nutrient supply becomes the main effect. This International Standard does not provide numerical values for the nutrients.

3.6
effect concentration
EC_x
concentration (mass fraction) of a test chemical or the percentage (mass fraction) of a test soil at which a given endpoint is inhibited by x % compared to the control

NOTE The effect concentration is expressed in milligrams per kilogram. When chemicals are tested, the EC_x is expressed as mass of the test substance per dry mass of soil; when soils are tested, the EC_x is expressed as a percentage of test soil dry mass per soil mixture dry mass.

3.7
emergence
development of a seedling contained within a seed, ending the latent period

NOTE It is expressed as the percentage of seedlings which emerge from test pots as compared with the control pots.

3.8
habitat function
ability of soils/soil materials to serve as a habitat for microorganisms, plants, soil-living animals and their interactions (biocenosis)

[ISO 15799]

3.9
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]

3.10
lowest observed effect concentration
LOEC
lowest tested concentration (mass fraction) of a test substance in soil at which a statistically significant effect on a given endpoint ($p < 0,05$) compared with the control is observed

cf. **NOEC** (3.11)

NOTE Analogously, the term LOEC is used for the lowest tested mixture ratio of a test soil in a reference or a standard control soil at which a statistically significant effect is observed. The LOEC is expressed as mass of the test substance per mass of dry soil or, in the latter case, as percentage of test-soil dry mass per soil-mixture dry mass. All test concentrations above the LOEC have a harmful effect equal 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 have been selected.

3.11
no observed effect concentration
NOEC
test substance concentration (mass fraction) or soil mixture ratio immediately below the LOEC, which when compared to the control has no statistically significant effect ($p < 0,05$)

cf. **LOEC** (3.10)

3.12
reference soil
uncontaminated site-specific soil (e.g. collected in the vicinity of a contaminated site) with properties (nutrient concentrations, pH, organic carbon content and texture) similar to the test soil

3.13
soil mixture ratio
ratio of the dry mass of test soil to the dry mass of reference/control soil

NOTE It is expressed as a percentage.

3.14
standard soil
field-collected soil or artificial soil whose main properties (e.g. pH, texture, organic matter content) are within a known range

EXAMPLES Euro soils, artificial soil.

NOTE The properties of standard soils may differ from those of the test soil.