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Soil quality — Effects of pollutants on mycorrhizal fungi — Spore germination test

Qualité du sol — Effets des polluants vis-à-vis des champignons mycorhizogènes — Essai de germination des spores

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Foreword

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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

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An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 10832 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

The corrected version of ISO/TS 10832:2009 incorporates the following corrections.

— Clause 1, last line: “mix” was changed to “mixture and sludge”.

— Subclause 3.12: the term “mass fraction” was changed to “inhibition concentration” and the symbol w_x was changed to IC_x; “mass fraction” was changed to “concentration” in the definition; a note was added.

— In addition, “mass fraction” was changed to “concentration” or “inhibition concentration” in 4.1, Clause 6, 7.4, 8.1 and 8.2 as well as in Annexes B, C and D; the symbol w_x was changed to IC_x (or the symbol w_{50} was changed to IC_{50}) in 4.1, 5.4 (modified), 8.1 and in Annex C.

— Subclause 5.2.4: the last sentence was changed.

— Subclause 5.3, first line: “the” was deleted.

— Subclause 5.5, Figure 1 a): the figure was replaced.

— Subclause 5.6.5: “fine” was changed to “thin”.

— Subclause 7.1.2: the last line was changed.

— Subclauses 7.2.2, 7.2.3, 7.2.4, 7.2.5: the last two sentences were changed.

— Subclause 7.3: the first line was changed. In Figure 2, “Petri dish” was changed to “Petri plate”.

— Subclause 7.3.4, 3rd and 4th lines: “assay” was changed to “test” and “retention” was changed to “holding”.

— Subclause 7.4.1, title and last line: “assay” was changed to “test”.

— Subclause 7.4.2: the title was changed.

— Subclause 7.6: the title and the last two sentences were changed.

— Subclause 8.1: the first sentence was changed.

— Subclause 8.2 was changed.

— Clause 9: the 2nd and 4th lines were changed. The last sentence was transferred to 5.4.

— Clause 10: Items b) and c) were changed.

— Annex B: the title was changed. In Table B.1, “assay” was changed to “test” and “mg/kg” was changed to “$mg \cdot kg^{-1}$”.

— Annex C: in Table C.1, “mg/kg” was changed to “$mg \cdot kg^{-1}$”.

Introduction

Mycorrhizal fungi are important components of the soil microbial community and key organisms in plant/soil systems. The root symbiosis they form represents a direct link between the soil and the large majority (80 %) of vascular plant species, in natural and agricultural environments. Mycorrhizal fungi provide several benefits to the host plant, including enhanced growth, improved mineral nutrition, greater drought resistance, and protection against pathogens and heavy metal stress.

Several studies have shown that mycorrhizal fungi are sensitive to pollutants such as metallic trace elements and polycyclic aromatic hydrocarbons, and to sewage sludges even when no phytotoxic effects on the host plant are observed. As mycorrhizal fungi fulfil most of the criteria for bioindicator organisms (ubiquitous in soil, sensitive to pollutants, ecologically relevant role in plant health and ecosystems), it appeared important to take them into account in hazard and environmental risk assessments linked to pollutants, contaminated soils and to the use of sewage sludge in agriculture.

Spore germination by an arbuscular mycorrhizal fungus, *Glomus mosseae*, makes up the basis of the proposed test. The first step of the symbiosis is taken into account in this test, whereas another test based on root colonization of the host plant is also under investigation.

This test can be directly performed with sludges or soils without any extraction step.

Soil quality — Effects of pollutants on mycorrhizal fungi — Spore germination test

1 Scope

This Technical Specification specifies a method to evaluate the effects of pollutants on spore germination of a mycorrhizal fungus, *Glomus mosseae*. This direct acute toxicity bioassay allows the evaluation of potential effects of pollutants and contaminated soils on beneficial soil microorganisms important for plant growth within the concept of sustainable agriculture.

This Technical Specification is applicable to

— chemical substances, and

— contaminated soils, waste and soil-waste mixture and sludge.

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 11263, *Soil quality — Determination of phosphorus — Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution*

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

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, definitions and abbreviated terms

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

3.1
mycorrhizal fungus
ubiquitous microorganism forming symbiotic association with the roots of vascular plant species

3.2
BEG
The International Bank for the Glomeromycota

3.3
spore
asexual reproductive unit of a fungus

3.4
sporocarps
mycelium-surrounded spore group

3.5
mycelium
branched hyphae network

3.6
hyphae
filaments which compose fungus mycelium

3.7
control substrate
inert substrate, which does not affect spore germination, used as a control or dilutant

3.8
matrix
test soil, sludge or waste

3.9
sandwich
device composed of two nitrocellulose membrane filters containing the spores

NOTE The two membranes are held together with two slide frames.

3.10
trypan blue staining
non-vital staining with trypan blue used to make mycorrhizal fungus structures visible (coloured in blue)

3.11
test mixture
mixture of test substance or matrix with a control substrate

3.12
inhibition concentration
IC_x
concentration of test substance or matrix inducing x % spore-germination inhibition compared to the control

NOTE "Mass fraction" is often known in biological methods as "concentration".

4 Test methods

4.1 Principle

Spores of *Glomus mosseae* are placed between two nitrocellulose membrane filters forming a sandwich (3.9), which is placed in a Petri dish containing the test mixture (3.11) which contains the test substance or matrix (3.8) with different concentrations, diluted or not with the control substrate (3.7).

The percentage of germinated spores is determined after 14 days.

The results are compared with a control substrate and used to estimate the 50 % spore-germination-inhibition concentration (IC_{50}).

Determination of another IC_x is also possible, but not required.

NOTE This method can be used to determine the effect of a single concentration.

4.2 Standard conditions

Use a growth chamber or room with a controlled temperature, (24 ± 2) °C.

Incubation is carried out in the dark (a darkroom or Petri dishes covered with aluminium foil).

5 Test materials

5.1 Distilled water

The pH of distilled water should be neutral and never less than 5,5.

5.2 Biological material

5.2.1 Fungus

Taxonomic group: Eumycota, Glomeromycota order.

Species: *Glomus mosseae* (Nicolson and Gerdeman) Gerdeman and Trappe (BEG 12).

5.2.2 Life status

Use mature spores.

5.2.3 Identification

Genbank identification number: U96139 (18s rDNA sub-unit); YO7656 (partial sequence of the 25s rDNA sub-unit) (25, 26).

5.2.4 Material

Use sporocarps (3.4) containing spores (3.3) (see Figure 1) purchased commercially[1)]. Use pot cultures that are less than five months old to extract sporocarps.

The spore-germination percentage shall be higher than 75 %. Conserve the spores and sporocarps in distilled water (5.1) at 4 °C.

The sporocarps shall be used within one week, and the spores within two days.

5.3 Control substrate

Use sand, or artificial soil in accordance with ISO 11268-1 as control substrate (3.7).

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1) Sporocarps of the fungus *Glomus mosseae*, produced and distributed by BioRize, are an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Use sand: > 99 % silica, pH 6,6 to 7,5, particle size 0,8 mm to 1,6 mm, washed three times with distilled water (pH > 6) (5.1), then dried. The final pH shall be > 6 (see ISO 10390).

Check that spore germination in the control substrate before setting up the bioassay is > 75 %.

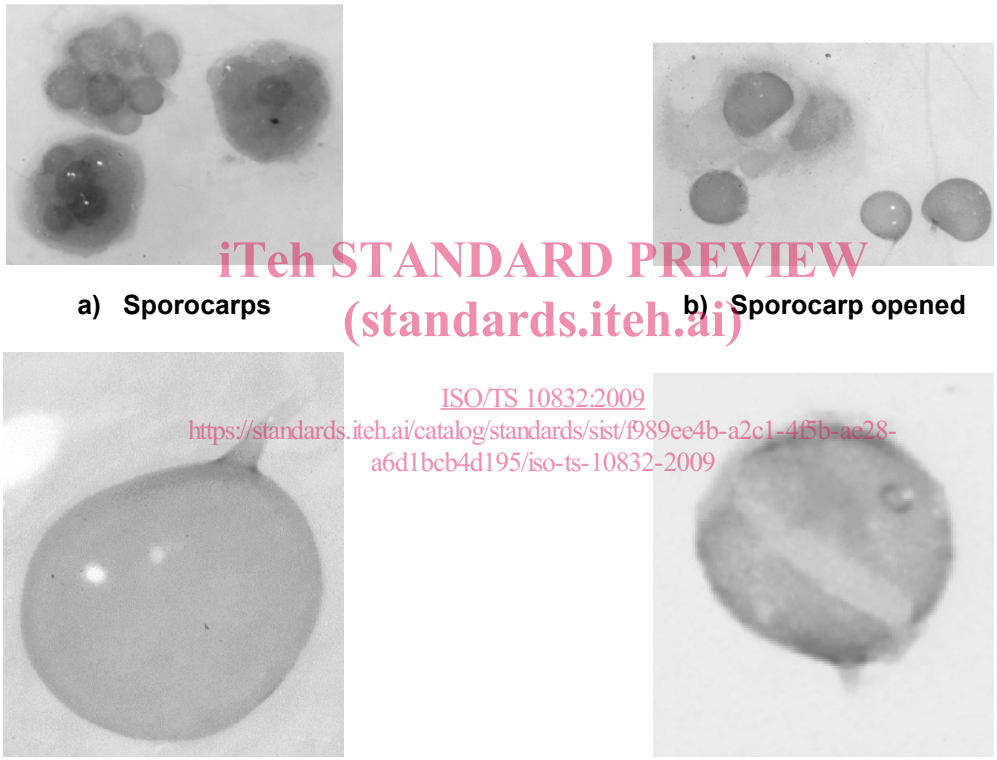
5.4 Reference substance

Use cadmium nitrate ($CdNO_3 \cdot 4H_2O$).

For information, in a ring test (n = 4) performed with cadmium nitrate, the value of IC_{50} in sand was between 0,15 mg·kg^{-1} and 1,7 mg·kg^{-1}.

5.5 Trypan blue

Use Trypan blue[2)], 0,5 g in 50 ml of HCl (1 %), 450 ml of H_2O, and 500 ml of glycerol.



a) Sporocarps b) Sporocarp opened

c) Intact spore d) Broken spore

Figure 1 — Sporocarps and spores of *Glomus mosseae*

5.6 Apparatus

5.6.1 Binocular microscope, 32 × magnifications.

5.6.2 Sterile plastic Petri dishes, of diameter 9 cm.

5.6.3 Slide frames, 24 mm × 36 mm.

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2) Trypan blue referenced 93595, distributed by Sigma Aldrich, is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

5.6.4 **Nitrocellulose membrane filters**, of diameter 47 mm, and porosity 0,45 µm, white, 3 mm gridlined.

5.6.5 **Ultra-thin nippers/tweezers**[3)].

5.6.6 **Filter paper**.

5.6.7 **Plastic microtube**, of capacity 1,5 ml.

5.6.8 **Plastic film**.

5.6.9 **Micropipette** and **cut tips**.

5.6.10 **Balance**, able to weigh from 0 g to 200 g, with an accuracy of 0,001 g.

5.6.11 **Funnel**.

6 Storage and preparation of samples

Soil samples shall be stored as specified in ISO 10381-6. Waste and sludge samples are stored at (4 ± 2) °C in tight containers. Containers used for microbiologically active sludge and waste should not be filled completely.

The following parameters should be determined for the soils, wastes and sludges to be tested:

— pH (see ISO 10390); use soil with a pH not lower than 5.5 (see Reference [18] in the Bibliography);

— water content (see ISO 11465);

— water-holding capacity (see ISO 11274);

— available phosphorus content (see ISO 11263);

— concentration of soluble phosphorus which shall be below 100 $mg \cdot kg^{-1}$ (see References [1], [2], [7] and [23]).

Use soils or wastes with a particle size below 4 mm in order to perform the bioassay. Otherwise, wastes should be ground and sieved to 4 mm, and soils should be sieved to 4 mm before preparing the test mixture.

7 Procedure

7.1 Biological system

7.1.1 Spore control

Glomus mosseae spores (3.3) should be yellow and bright, and have an intact and clean envelope (empty and crushed spores should be eliminated), see also Figure 1.

7.1.2 Preparation of the biological material

Number of individual spores for each concentration tested: 30 spores (3.3) per sandwich (3.9), six replicates (sandwiches) per concentration; i.e. 180 spores per concentration.

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3) Ultra-thin tweezers referenced T5130 No. 5, distributed by Oxford Instruments, are an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.