



SLOVENSKI STANDARD
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Kakovost tal - Vzorčenje nevretenčarjev v tleh - 4. del: Vzorčenje, ekstrakcija in identifikacija nematod iz tal (ISO/DIS 23611-4:2021)

Soil quality - Sampling of soil invertebrates - Part 4: Sampling, extraction and identification of soil-inhabiting nematodes (ISO/DIS 23611-4:2021)

Bodenbeschaffenheit - Probenahme von Wirbellosen im Boden - Teil 4: Probenahme, Extraktion und Bestimmung von Boden bewohnenden Nematoden (ISO/DIS 23611 4:2021)

Qualité du sol - Prélèvement des invertébrés du sol - Partie 4 : Prélèvement, extraction et identification des nématodes du sol (ISO/DIS 23611-4:2021)

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Soil quality — Sampling of soil invertebrates —

Part 4: Sampling, extraction and identification of soil-inhabiting nematodes

*Qualité du sol — Prélèvement des invertébrés du sol —**Partie 4: Prélèvement, extraction et identification des nématodes du sol*

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*.

This second edition cancels and replaces the first edition (ISO 23611-4:2007), which has been technically revised. The main change to the previous edition is as follows:

- addition of examples of nematods monitoring programmes (including presentation of their results) as an informative annex.

A list of all parts in the ISO 23611 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document has been drawn up since there is a growing need for the standardization of terrestrial zoological field methods. Such methods, mainly covering the sampling, extraction and handling of soil invertebrates, are necessary for the following purposes:

- biological classification of soils including soil quality assessment [36],[41],[56];
- terrestrial bio-indication and long-term monitoring [24],[27],[30],[49];
- evaluation of the effects of chemicals on soil animals (ISO 11268-3[33]).

Data for these purposes are gained by standardized methods since they can form the basis for far-reaching decisions (e.g. whether a given site should be remediated or not). In fact, the lack of such standardized methods is one of the most important reasons why bio-classification and bio-assessment in terrestrial (i.e. soil) habitats has so far been relatively rarely used in comparison to aquatic sites.

Nematodes are an important and major part of the soil fauna. Some authors estimate that this group is probably the most dominant one of the multicellular organisms (Metazoa) on earth[51]. Nematodes occur from the Antarctic to the tropics and from deep sea sediments to mountain regions. They are active in every place with sufficient water and organic material. The species diversity and functional variety are impressive[14]. Nematodes are commonly known as parasites of animals and plants, but the major part of the nematode fauna participates in decomposition processes by feeding on bacteria and fungi.

Nematodes occur in high numbers ($0,2 - 9 \times 10^6 \text{ m}^{-2}$) and with a high (10 to 100) species diversity in almost every soil sample[12]. Moreover there is a broad ecological spectrum of feeding types and food web relations among the nematodes such as bacterivores, fungivores, herbivores, predators and omnivores[56],[57]. These factors make the group highly suitable as indicators for ecological soil quality[55], but standardization of methods is urgently needed for comparison and combination of results.

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In the past 100 years, nematology has developed strongly from the viewpoint of agriculture, advisory sampling and phytosanitary regulations because some terrestrial nematodes cause a lot of damage in crops. With respect to methods, there are several “schools” in different parts of the world with their own history, practical advantages and disadvantages. A comprehensive overview is given by Oostenbrink[14] and Southey[47],[48]. The more recently described methods (or variants) are often developed with special interest to certain plant parasitic species. Within the past 20 years new methods have evolved that allow a DNA-based taxonomic identification of nematode species[20],[33],[53]. This opens the taxonomic analysis of nematodes to a broader community of non-specialists.

Since Bongers[16] introduced the Maturity Index, the use of nematodes in bio-indication for soil quality has increased rapidly[55]. Nematodes are now used for ecological soil research and monitoring in several countries all over the world. Monitoring activities make special demands on methodology, for instance, that a large number of soil samples is processed on a routine basis against reasonable costs. Some of the methods originally developed for advisory sampling in agriculture are very suitable for ecological research. They form the basis for specific variants described in this document.

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Soil quality — Sampling of soil invertebrates —

Part 4:

Sampling, extraction and identification of soil-inhabiting nematodes

1 Scope

This document specifies a method for sampling and handling free-living nematodes from terrestrial field soils as a prerequisite for using them as bio-indicators (e.g. to assess the quality of a soil as a habitat for organisms).

This document applies to all terrestrial biotopes in which nematodes occur. The sampling design of field studies in general is specified in ISO 18400-101.

This document is not applicable to aquatic nematodes because of differences in the sample matrix (e.g. water column). Methods for some other soil organism groups such as earthworms, collembolans, enchytraeids or macro-invertebrates are covered in ISO 23611-1, ISO 23611-2, ISO 23611-3 and ISO 23611-5.

The nematodes that are characterized by the proposed procedure are all the free-living forms of nematodes found in soil. They include non-plant-feeding nematodes as well as ectoparasitic plant-feeding nematodes and free-living stage of endoparasitic nematodes. The quantification of obligate plant-feeding nematodes in roots requires specific methods!

NOTE Basic information on the ecology of nematodes and their use as bio-indicators can be found in the bibliography.

This document does not cover the pedological characterization of the site which is highly recommendable when sampling soil invertebrates. ISO 10390, ISO 10694, ISO 11272, ISO 11274, ISO 11277, ISO 11461 and ISO 11465 include suitable procedures for measuring pH, particle size distribution, C/N ratio, organic carbon content and water-holding capacity.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp/>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1

nematode

small, non-segmented free-living worm (up to a few millimetres in length) belonging to the class Nematoda

Note 1 to entry: Nematodes without a soil-inhabiting stage are not included in this context.

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3.2

location

study area or plot that is characterized based on the composition of (among others) the nematode fauna

3.3

bulk-sample

composite soil sample made out of many small soil cores to get an impression of the average nematode composition

3.4

soil sampler

tool to collect soil material in a quick and standardized way

3.5

Oostenbrink elutriator¹⁾

metal funnel with an upward water flow to separate nematodes from larger soil particles

See [Figure A.3](#).

3.6

mass slide

microscopic slide on which 300 to 400 nematodes are mounted for species identification

3.7

identification

determination of the species, genus or family of an individual based on morphological characteristics (mouth parts, sexual organs, body ratios) with an identification key

3.8

colonizer – persister (cp) scale

ecological classification of nematodes, proposed by Bongers^{[16],[17]}

Note 1 to entry: The principle is analogous to the r-K life strategies during succession, distinguished in fundamental ecology. Non-plant-feeding nematode families are classified to one of the five cp-groups. This is also the basis for the calculation of the Maturity Index.

4 Principle

Nematodes are collected in soil samples with a small cylindrical core (diameter: circa 2 cm; length: 10 cm) or an auger (see [Figure A.2](#)). For monitoring purposes, the soil samples are combined in a bulk-sample from a homogeneous area. The total number of samples to be taken depends on the investigated surface area and its homogeneity (e.g. pedology, and use, crop). The individual samples can be gathered in the field in a standard plastic bag or plastic bucket. The combined bulk-sample is too large for direct examination and therefore it is mixed and subsampled. In the field and during transport to the laboratory, the soil samples shall be protected against strong fluctuations in temperature, water-loss and heavy mechanical disturbance. They can be stored for at most four weeks at 4 °C.

NOTE 1 The sampling method described above is derived from “the Dutch Method” ^[48] for determining the infestation of a field with potato-cyst nematodes, and has been used for many years in several European countries.

The Oostenbrink funnel method is recommended for routine extractions of soil samples, for instance in a monitoring network. The Oostenbrink method is not the most simple one that can be used under any circumstance. However, it has several advantages: it is highly standardized and constant in extraction efficiency. The Oostenbrink wet funnel method combines three basic means that can be used for the

1) Oostenbrink elutriator is the trade name of a product supplied e.g. by MEKU Erich Pollähne GmbH (<http://www.meku-pollaehne.de/Nematologie/Oostenbrink-Elutriator/oostenbrink-elutriator.html>). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

separation of nematodes from soils: washing, sieving, active movement. Therefore, it obtains better results than any one of the basic methods individually. Further advantages are given below:

- relatively large soil samples of any soil type can be treated at once (100 g to 500 g);
- clean nematode suspensions;
- isolation of most living and active nematodes;
- there are many years of experience with enormous amounts of routine soil extractions;
- it is used in many places around the world.

After sampling, the nematodes are extracted from the soil using the Oostenbrink elutriator 1) (model III) (see [Figure A.3](#) and [Annex B](#)). In this technique, an upward current of water separates the nematodes from soil particles and holds them in suspension while the heavier particles sink^{[10],[32],[43],[48]}. This suspension of nematodes and small particles passes through three sieves (mesh width: 45 µm). The catch is washed from the sieves onto a cotton-wool filter (milk filter). The cotton-wool filter is mounted on a supporting sieve and is placed in a dish with 100 ml of tap water. For three days, through their active downwards movement, the nematodes separate themselves from the debris on the filter. Thus, the living nematodes actively crawl through the filter in a dish with tap water.

After extraction, the nematodes are counted in 2 × 10 % of the 100 ml suspension, then concentrated, preserved and mounted on mass slides. Finally, at least 150 individuals or a fixed percentage of the total number in the sample is identified under the microscope.

Mature nematodes can be identified to species level. However, populations in the soil are often dominated by juveniles and the genera level of taxonomy is a practical (but less sensitive) way of distinction.

Alternative extraction methods such as the Seinhorst elutriator^[43], Baermann funnel ([Annex C](#)) can be useful under special circumstances, but are not recommended as general procedures because the Oostenbrink elutriator is robust, easy to operate and usually quantitatively superior to most other techniques. As an alternative, flotation extraction using colloidal silica are recommended for preserved samples containing non-living organisms^[25]

NOTE 2 This part of ISO 23611 is not applicable for aquatic nematodes because these nematodes do not pass through the filter. Special centrifugation techniques are available for sediment samples.

NOTE 3 Determination with a light microscope is based on morphological characteristics. In some cases, it is not possible to recognize the specimen on species or even genus level, e.g. juveniles. New techniques, such as DNA barcoding or metabarcoding^{[39],[53]}, offer a morphology-independent taxonomy, so that also juveniles can be identified to genus or species level.

NOTE 4 The sampling of nematods is often included in much broader monitoring programmes which try to cover the whole soil fauna or parts of it (e.g. the mesofauna). Examples of the use of soil invertebrates are given in [Annex D](#). The design of such programmes is not included in this document.

5 Reagents

5.1 **Formalin** [formaldehyde solution, 6 % (volume fraction)].

5.2 **Paraffin**, with melting point near 60 °C.

6 Apparatus

Use standard laboratory equipment and the following.

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6.1 Sampling

6.1.1 Soil sampler, of an open, closed or split-tube type.

EXAMPLE Grass plot sampler (diameter: 23 mm) or soil auger ([Figures A.2](#) and [A.3](#)); commercially available.

6.1.2 Plastic bucket (collection of soil samples in the field).

6.1.3 Plastic container, for mixing of the bulk-sample.

6.1.4 Sieve, with 8 mm apertures.

6.1.5 Coated bags or plastic bags or glass vessels (transport and storage).

6.1.6 Permanent marker or pre-printed labels.

6.2 Extraction

6.2.1 Beaker, of capacity 100 ml to 250 ml.

6.2.2 Balance, able to weigh 1 kg to 25 kg, for weighing the total sample mass.

6.2.3 Oostenbrink elutriator¹⁾ (see also [Annex B](#)).
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6.2.4 Three sieves, with 45 µm apertures and 30 cm diameters.

6.2.5 One sieve, with 250 µm apertures and a 10 cm diameter.
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6.2.6 Plastic bowl, of capacity circa 2 l.

6.2.7 Clamping ring.

6.2.8 Extraction sieve, with 1 000 µm apertures and 16 cm diameter.

6.2.9 Milk- or cotton-wool filters.

6.2.10 Shallow trays (Petri dishes) or **special extraction dishes**.

6.2.11 Glass vessel, of capacity 100 ml, with a screw-cap.

6.3 Counting

6.3.1 Dissecting microscope, 10× to 50× magnification.

6.3.2 Small counting dish with grid or glass slide with grid.

NOTE Counting dishes in several sizes and different grids are available from the manufacturers of laboratory equipment. They can also be made out of small plastic Petri dishes by scratching a grid on the bottom with a needle.

6.3.3 Simple hand counting device.