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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

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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.

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ISO 23611-4 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

ISO 23611 consists of the following parts, under the general title *Soil quality — Sampling of soil invertebrates*:

— *Part 1: Hand-sorting and formalin extraction of earthworms*

— *Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina)*

— *Part 3: Sampling and soil extraction of enchytraeids*

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

Introduction

This part of ISO 23611 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 [15],[17],[28];

— terrestrial bio-indication and long-term monitoring [9],[10],[13],[24];

— evaluation of the effects of chemicals on soil animals (ISO 11268-3).

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. 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. 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 [(5 000 to 100 000)/kg fresh soil] and with a high (20 to 100) species diversity in almost every soil sample. 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 [27],[28]. These factors make the group highly suitable as indicators for ecological soil quality, but standardization of methods is urgently needed for comparison and combination of results.

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 [22],[23]. The more recently described methods (or variants) are often developed with special interest to certain plant-parasitic species.

Since Bongers [4] introduced the Maturity Index, the use of nematodes in bio-indication for soil quality has increased rapidly. 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.

Soil quality — Sampling of soil invertebrates —

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

1 Scope

This part of ISO 23611 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 part of ISO 23611 applies to all terrestrial biotopes in which nematodes occur. The sampling design of field studies in general is specified in ISO 10381-1.

This part of ISO 23611 is not applicable to aquatic nematodes because these nematodes do not pass through the filter. Methods for some other soil organism groups such as earthworms, enchytraeids or collembolans are covered in other parts of ISO 23611.

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 part of ISO 23611 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 are more suitable for measuring pH, particle size distribution, C/N ratio, organic carbon content and water-holding capacity.

2 Terms and definitions

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

2.1
nematode
small, non-segmented free-living worm (up to a few millimetres in length) belonging to the class Nematoda

NOTE Nematodes without a soil-inhabiting stage are not included in this context.

2.2
location
study area or plot that is characterized based on the composition of (among others) the nematode fauna

2.3
bulk-sample
composite soil sample made out of many small soil cores to get an impression of the average nematode composition

2.4
soil sampler
tool to collect soil material in a quick and standardized way

2.5
Oostenbrink elutriator [1)]
metal funnel with an upward water flow to separate nematodes from larger soil particles

See Figure A.3.

2.6
mass slide
microscopic slide on which 300 to 400 nematodes are mounted for species identification

2.7
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

2.8
colonizer – persister (cp) scale
ecological classification of nematodes, proposed by Bongers [4],[5]

NOTE 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.

3 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, culture). 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” [23] for determining the infestation of a field with potato-cyst nematodes, and has been used for many years in several European countries.

1) Oostenbrink elutriator is the trade name of a product supplied by firm Eijkelkamp, Giesbeek, NL (http://www.eijkelkamp.nl). 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.

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 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 [1],[14],[19],[23]. 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 [19] or 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, centrifugation techniques are most suitable.

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 level, e.g. juveniles. With a new technique based on DNA analysis, juveniles can be identified to species level. This new technique is expected to become operational within several years.

4 Reagents

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

4.2 Paraffin, with melting point near 60 °C.

5 Apparatus

Use standard laboratory equipment and the following.

5.1 Sampling

5.1.1 **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.

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

5.1.3 **Plastic container**, for mixing of the bulk-sample.

5.1.4 **Sieve**, with 8 mm apertures.

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

5.1.6 **Permanent marker** or **pre-printed labels**.

5.2 Extraction

5.2.1 **Beaker**, of capacity 100 ml to 250 ml.

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

5.2.3 **Oostenbrink elutriator** [1] (see also Annex B).

5.2.4 **Three sieves**, with 45 µm apertures and 30 cm diameters.

5.2.5 **One sieve**, with 250 µm apertures and a 10 cm diameter.

5.2.6 **Plastic bowl**, of capacity circa 2 l.

5.2.7 **Clamping ring**.

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

5.2.9 **Milk-** or **cotton-wool filters**.

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

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

5.3 Counting

5.3.1 **Dissecting microscope**, 10× to 50× magnification.

5.3.2 **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.

5.3.3 **Simple hand counting device**.

5.3.4 **Aquarium pump**, for mixing nematode suspensions.

5.3.5 **Pipette** (drop glass), with adjustable volume.

5.3.6 **Handling needle**.

5.3.7 **Bottle**, of volume 100 ml.

5.4 Fixation and preparation of mass slides

5.4.1 Water jet pump, for concentration of suspension.

5.4.2 Glass slides, 50 mm × 76 mm.

5.4.3 Cover glasses, 45 mm × 45 mm.

5.4.4 Electric heating plate.

5.4.5 Metal stamp, 40 mm × 40 mm, for paraffin seal on glass slides.

5.5 Identification

5.5.1 Microscope, magnification 400× to 1 000×.

5.5.2 Ocular micrometer indicator.

5.5.3 Identification keys [3].

5.5.4 Standard form, to list the identification results.

6 Procedure

6.1 General

For quality assurance, each sample shall be given a unique code from the moment it is taken in the field. This code (label) shall stay with the sample during all the processing and analysis steps. Standard (electronic) form(s) should be used to follow the routing of the samples and collection of analysis results. These basic data may be combined in a spreadsheet or database file for further calculations and statistical testing.

6.2 Sampling

While the density and diversity of soil nematodes are the highest in the top 10 cm of the mineral soil, a grass plot sampler (5.1.1) with a 10 cm or 15 cm long sampling-tube is appropriate for most biomonitoring purposes. It is recommended to use a closed tube with a fixed length and diameter.

EXAMPLE 1 A grass plot sampler consists of a stainless steel gouge auger (available in different dimensions) consisting of a steel auger pipe, a collecting bucket (5.1.2) and a stick with a steel handle. Because of the conical shape of the pipe, the sample is easily pushed toward the collecting bucket when the next sample is taken. The sample depth is constant and soil cores can be collected easily over a large area (see Figure A.1). This device can be used in many situations.

EXAMPLE 2 Alternatively, a soil auger can be used as a simple, cheap and quick working device. Augers are available in different diameters. Soil samples collected with an auger are less compressed. The disadvantage is that soil material can be lost more easily (see Figure A.2).

EXAMPLE 3 When accurate separation of soil layers is required, a split-tube sampler can be used. This sampling device needs more handling time and is less suited for large numbers of samples and large areas (see Figure A.2).

Samples from deeper layers can be taken with an auger to avoid excessive soil compression, or special split-tube samplers (see Figure A.2). Organic or litter material can be included in the samples, but it increases the numbers of nematodes found, sometimes considerably. Organic layers may be sampled independently. In this case, a wider split-tube corer (5 cm to 10 cm) is preferred in order to separate the organic horizons from the mineral material. Small amounts of litter can also be treated in an Oostenbrink elutriator [1)] (5.2.3) to extract the nematodes. Extraction efficiency can be enhanced by soaking and blending the organic parts [16],[18].