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Soil quality — Sampling of soil invertebrates —

Part 3: Sampling and extraction of enchytraeids

Qualité du sol — Prélèvement des invertébrés du sol — Partie 3: Prélèvement et extraction des enchytréides

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Foreword

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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 23611-3:2007), which has been technically revised. The main changes to the previous edition are as follows:

— addition of examples of enchytraeid 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 developed to address a growing need for the standardization of terrestrial zoological field methods. Such methods, mainly covering the sampling, extraction and handling of soil invertebrates, are needed for the following purposes:

— biological classification of soils including soil quality assessment (e.g. References [4], [25], [27], [31], [36]);

— terrestrial bioindication and long-term monitoring (e.g. References [4], [30]);

— evaluation of the effects of chemicals on soil animals (References [18], [26], [28]).

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 biological classification concepts in terrestrial (i.e. soil) habitats have so far been relatively rarely used in comparison with aquatic sites.

Originally, the methods described here were developed for taxonomical and ecological studies, investigating the role of enchytraeids in various soil ecosystems. These animals without doubt belong to the most important soil invertebrates in temperate regions (mainly in acidic soils[7]). Their influence on soil functions like litter decomposition and nutrient cycling is well known[17][23]. Due to their often very high numbers, and their population biomass, they are also important in many terrestrial food-webs[6]. Some species have unintentionally been distributed by humans in many soils of the world.

Since it is neither possible nor useful to standardize methods for all soil organisms, the most important ones have been selected. Microbiological parameters are already covered by existing ISO standards (e.g. ISO 14240-1, ISO 14240-2, ISO 17601, ISO/TS 29843-1 and ISO/TS 29843-2.

Soil quality — Sampling of soil invertebrates —

Part 3:
Sampling and extraction of enchytraeids

1 Scope

This document specifies a method for sampling, handling and extracting enchytraeids from terrestrial field soils as a prerequisite for using these animals as bioindicators (e.g. to assess the quality of a soil as a habitat for organisms).

Basic information on the ecology of enchytraeids and their use as bioindicators in the terrestrial environment is included in the Bibliography.

This document applies to all terrestrial biotopes in which enchytraeids occur. The sampling design of field studies in general is given in ISO 18400-101. These details can vary according to the climatic/regional conditions of the site to be sampled and an overview on the determination of effects of pollutants on enchytraeids in field situations is given in Reference [6].

Methods for some other soil organism groups such as earthworms or arthropods are given in ISO 23611-1, ISO 23611-2, ISO 23611-4 and ISO 23611-5.

This document is not applicable for very wet or flooded soils and might be difficult to use under extreme climatic or geographical conditions (e.g. in high mountains).

When sampling soil invertebrates, it is highly recommendable to characterize the site (e.g. concerning soil properties, climate and land use). However, such a characterization is not covered by this document. 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 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 http://www.electropedia.org/

3.1
enchytraeid
small soil-inhabiting worm (a few millimetres to several centimetres in length) belonging to the family Enchytraeidae, class Oligochaeta, superclass Clitellata, phylum Annelida

Note 1 to entry: The common name for enchytraeid is potworm[35].

EXAMPLE Species of the genera *Enchytraeus, Fridericia* or *Cognettia*.

4 Principle

Enchytraeids at a certain site are sampled from the soil by using a split soil corer (diameter usually 3 cm to 6 cm) (6.1). After sampling, the soil samples containing the enchytraeids are transported to the laboratory. Then the enchytraeids are extracted from soil by means of a wet extraction method. (This approach has been well known for a long time[12][20][24]) After extraction, the enchytraeids are identified alive and, if required, preserved in such a way that they can be stored in a collection indefinitely (e.g. for taxonomical purposes).

The determination of the biomass of enchytraeids is also described in this document. The abundance and biomass values can be recalculated to the area of the soil corer or, more rarely, volume parameters.

NOTE 1 The sampling of enchytraeids 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 B. The design of such programmes is not included in this document (but see e.g. Reference [3]).

NOTE 2 Some hints for the taxonomy of enchytraeids are given in the Bibliography.

5 Reagents

5.1 Tap water (without toxic properties, e.g. due to copper contamination).

5.2 Ethanol, 70 % (volume fraction).

5.3 Bengalred, 4,5,6,7-Tetrachloro-2',4',5',7'-tetraiodofluorescein formulated as a staining agent.

5.4 Bouin's fixative, buffered solution of formaldehyde, acetic acid and picric acid.

5.5 Paracarmin, staining agent, prepared as a mixture of carmine acid, aluminium chloride and calcium chloride solved in ethanol.

5.6 Canada-balm, natural yellowish viscous fluid containing 13 % to 14 % (volume fraction) Canadin acid ($C_{20}H_{38}O_2$), 48 % to 50 % (volume fraction) α- and β-Canadinol acid ($C_{19}H_{30}O_2$) and 5 % (volume fraction) Canadoesen ($C_{21}H_{40}O$).

6 Apparatus

6.1 Split soil corer (e.g. diameter 3 cm to 6 cm; extracted core length 10 cm to 30 cm); length in total variable (depending whether or not a handle is used) and a plastic or wooden impact-absorbing hammer.

6.2 Plastic bags (e.g. 1-l freezer bags); general store.

6.3 Temperature recorder or a **minimum/maximum-thermometer**.

6.4 Plastic bowls, diameter approximately 20 cm, height approximately 10 cm; general store.

6.5 Plastic sieves, diameter approximately 15 cm, mesh width approximately 1,0 mm; general store.

6.6 60 W bulbs as a heating device; general store.

6.7 Glassware, for example petri dishes (square format) with a size of 8 cm × 8 cm or small glass vessels (e.g. 50 ml).

6.8 Large, sharp knife.

6.9 Refrigerator.

6.10 Dissecting microscope with low magnification (10 times to 40 times).

6.11 Microscope with high magnification (60 times to 400 times) and equipped with an interference lighting device.

6.12 Spring steel pincers (flat).

6.13 Transfer tool, pasteur pipette, soft steel forceps or a hooked needle.

7 Procedure

7.1 Soil sampling

The soil samples to be used for the investigation of the enchytraeid community are taken destructively by means of a split soil corer (6.1). The corer is carefully pressed into the soil. The depth depends on the land use and soil type, but usually varies from 10 cm (e.g. forests) up to 30 cm (e.g. crop sites), i.e. those layers in which the bulk of the enchytraeids are living. In rare cases, for example if thick roots are present, a plastic or wooden impact-absorbing hammer can be used to take the samples. After removing the soil corer, its valve is opened and the soil core is carefully taken out by hand. The core is divided into cylinders (e.g. 3 cm to 4 cm in height) with a knife (6.8). These soil cylinders may be stored in small plastic bags (6.2) in a refrigerator (6.9) at approximately 4 °C to 6 °C for a period of preferably not longer than one week to two weeks (storage should not exceed one month in any case[9]). The soil corer is cleaned with water afterwards.

7.2 Extraction of the enchytraeids

In principle, the extraction of the worms from the soil is caused by their active movement through the water-saturated sample into the water-filled bowl (6.4).

The extraction should commence as soon as possible after the sampling (see 7.1). The bowls (6.4) are carefully filled up with tap water (5.1). The samples (i.e. soil cylinders) are put in the sieves, and are, if necessary (e.g. in cases of heavy loam soils), carefully broken apart by hand (see Figure 1). The samples in the sieve shall be completely submerged and the bottoms of the sieves should not reach the bottom of the bowls. To ensure an extraction efficiency of Enchytraeidae from the samples of more than 90 %, the extraction of soil should last for 2 d to 7 d and extraction of litter for 0,5 d to 2 d at (12 ± 2) °C (water temperature). The duration depends mainly on the organic content of the sample. These times can be modified according to organizational requirements and the number of individuals in a sample. However, the worms quickly die if an oxygen deficiency occurs. In order to avoid this problem, the water should be changed after 18 h to 24 h, and again after 48 h (if extraction period exceeds 2 d). For that purpose the sieve with the sample should be carefully transferred to a bowl with fresh water. An acceleration of the extraction using a heat source [e.g. a 60 W bulb (6.6)] placed above the sample can be helpful, but should be carefully used (i.e. slow temperature increase over at least 3 h), since otherwise — species-specifically — many animals, especially juveniles and fragmentation stages, remain in the soil (see Annex A).

NOTE 1 In order to reduce the amount of debris at the bottom of the extraction bowls, a fine wiping cloth (mesh size 1 mm) can be put in the sieve before the soil sample is put in.

At the end of the extraction procedure, the sieves are removed. The requirements for the disposal of the soil are given in the appropriate national regulations. The water is slowly and carefully decanted from the bowl. The finest fraction of soil at the bottom of the bowls should not be disturbed (see Figure 2). A small amount of water (up to a height of 5 mm to 10 mm) shall remain in the bowls. Subsequently, the

finest fraction of soil is suspended in the overlying water, placed in a petri dish (6.7) and briefly stored until soil particles have settled and the water becomes clear. Since the whitish worms are heavier than water, but are rarely able to hide themselves in the narrow soil layer, they can easily be collected out of the petri dish under a dissecting microscope (6.10). For this transfer, a soft steel forceps, a Pasteur pipette or a hooked needle (6.13) can be used, but in any case, damaging of the worms shall be avoided. The most convenient way of counting the total number is to divide the surface of the petri dish into parallel rows which are checked one after another. Due to their white colour, the worms are clearly visible against the usually brownish soil particles. The animals are transferred to small plastic or glass vessels (e.g. 20 ml).

The number of samples which can be extracted simultaneously is theoretically unlimited. However, due to the size of the water bowls, space limitations can occur. Since they (i.e. at least the water) shall be cooled, usually only up to 40 to 50 samples can be processed at one time. These limitations can be overcome by carrying out the procedure in a cool room, for example a cellar.

NOTE 2 In rare cases, the enchytraeids can be confused with Diptera larvae (which very often possess brownish or black head capsules) or nematodes (non-peristaltic movement; usually smaller and faster moving than oligochaetes). Additionally, fungal hyphae or fine root material can be mistaken for enchytraeids, since they can possess the same length and colour. However, they always lack the segmentation of oligochaete worms.

Figure 1 — Extraction bowl with soil sample

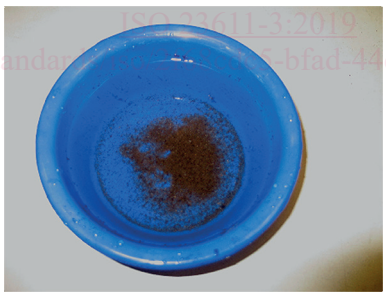

Figure 2 — Sediment layer (including enchytraeids)

7.3 Microscopic identification

The microscopic identification should be done as soon as possible, because the animals die in water after some days, even if stored in a refrigerator (6.9). A soft steel forceps, a Pasteur pipette or a hooked needle (6.13) can be used carefully to transfer the animals with a drop of water to a slide. If the worms are moving too fast on the slide, they can be anaesthetised with CO_2 (e.g. by using a drop of mineral water with gas, but it should be used with care, otherwise the worms are killed).

NOTE Identification of the enchytraeids is difficult. Therefore, in many cases only the number of animals is determined. Otherwise, keys for European enchytraeid species[20] and for species of the genus Fridericia[32], as well as other publications, are available. A compromise can be the use of a site-specific key, since usually only three to 25 species occur at any given site (in this case, often worms fixed in ethanol can be identified to the species level). An overview of the information (e.g. parameters, drawings) needed for the identification of a certain species is given in Reference [11].

