SLOVENSKI STANDARD
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Kakovost tal - Vzorčenje nevretenčarjev v tleh - 3. del: Vzorčenje in ekstrakcija enhitrej iz tal (ISO/DIS 23611-3:2018)

Soil quality - Sampling of soil invertebrates - Part 3: Sampling and extraction of enchytraeids (ISO/DIS 23611-3:2018)

Bodenbeschaffenheit - Probenahme von Wirbellosen im Boden - Teil 3: Probenahme und Bodenextraktion von Enchytraeen (ISO/DIS 23611-3:2018)

Qualité du sol - Prélèvement des invertébrés du sol - Partie 3: Prélèvement et extraction des enchytréides (ISO/DIS 23611-3:2018)

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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

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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The committee responsible for this document is ISO/XXX

This second/third/... edition cancels and replaces the first/second/... edition (), [clause(s) / subclause(s) / table(s) / figure(s) / annex(es)] of which [has / have] been technically revised.

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

This second edition cancels and replaces the first edition (ISO 23611-1:2006), which has been technically revised. The main changes are:

— the addition of examples of earthworm 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.

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 needed for the following purposes:

— biological classification of soils including soil quality assessment (e.g. References[36][25],[33],[4][27]);

— terrestrial bioindication and long-term monitoring (e.g. References[34],[15], ,[30],[4]);

— 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 to 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 number which is often very high (and to their population biomass), they are also important in many terrestrial food-webs [[6]]. Some species have unintentionally been distributed by man 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 29843-1, and ISO 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 are included in the Bibliography.

This document applies to all terrestrial biotopes in which enchytraeids occur. The sampling design of field studies in general is specified 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 specified in ISO 23611-1, ISO 23611-2, ISO 23611-4 and ISO 23611-5.

This document is not applicable for semi-terrestrial (i.e. living in or close to the pure water) 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 part of ISO 23611. 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.

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

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

2.1
enchytraeids
small soil-inhabiting worms (a few millimetres to several centimetres in length) belonging to the family Enchytraeidae, class Oligochaeta, superclass Clitellata, phylum Annelida) (common name: potworms[35])

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

3 Principle

Enchytraeids at a certain site are sampled from the soil by using a split soil corer (diameter usually 3 cm to 6 cm) (5.1). 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 [[13]], [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 part of ISO 23611. 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). The design of such programmes is not included in this part of ISO 23611 (but see e.g[3].).

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

4 Reagents

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

4.2 Ethanol, 70 % (volume fraction).

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

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

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

4.6 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 ($C_{21}H_{40}O$).

5 Apparatus

5.1 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.

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

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

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

5.5 Plastic sieves, diameter approximately 15 cm, mesh width approximately 0,5 mm; general store.

5.6 60-W bulbs as a heating device; general store.

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

5.8 Sharp, large knife.

5.9 Refrigerator.

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

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

5.12 Spring steel pincers (flat).

5.13 Transfer tool: Pasteur pipette, soft steel forceps or a hooked needle.

6 Procedure

6.1 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 (5.1). The corer is carefully pressed into the soil. The depth depends on the land use and soil type, but usually varies between 10 cm (e.g. forests) and up to 30 cm (e.g. crop sites), i.e. those layers in which the bulk of the enchytraeids are living. In rare cases, e.g. 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 height) with a knife (5.8). These soil cylinders may be stored in small plastic bags (5.2) in a refrigerator (5.9) at approximately 4 °C to 6 °C for a period of preferably not longer than one to two weeks (storage should not exceed one month in any case [[9]]). The soil corer is cleaned with water afterwards.

6.2 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 (5.4).

The extraction should commence as soon as possible after the sampling (see 6.1). The bowls (5.4) are carefully filled up with tap water (4.1) until the empty sieves (5.5) are completely covered by the water. 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 bottom 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 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 18-24 h, and again after 48 h (if extraction period exceeds 2 d).. An acceleration of the extraction using a heat source [e.g. a 60-W bulb (5.6)] 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 B).

NOTE 1 In order to reduce the amount of debris at the bottom of the extraction bowls, a fine wiping cloth (mesh size 0,5 mm) can be put in the sieve before the soil sample is put in [[31]].

At the end of the extraction procedure, the sieves are removed, the soil is discarded and disposed according to local waste 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 (5.7) 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 (5.10). For this transfer, a soft steel forceps, a Pasteur pipette, soft steel forceps or a hooked needle (5.13) 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