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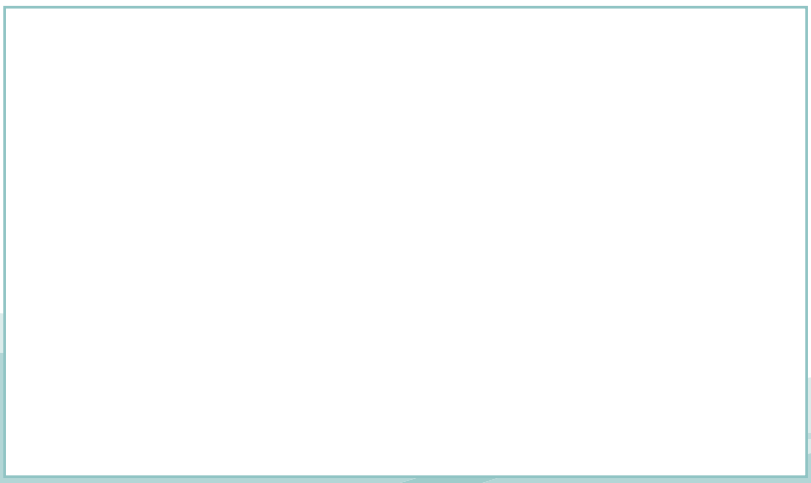
Markundersökningar – Provtagning av marklevande ryggradslösa djur –
Del 3: Provtagning och extraktion av enchytraeider (ISO 23611-3:2007)

Soil quality – Sampling of soil invertebrates –
Part 3: Sampling and soil extraction of enchytraeids (ISO 23611-3:2007)



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Europastandarden EN ISO 23611-3:2011 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 23611-3:2011.

Denna standard ersätter SS-ISO 23611-3:2008, utgåva 1.

The European Standard EN ISO 23611-3:2011 has the status of a Swedish Standard. This document contains the official version of EN ISO 23611-3:2011.

This standard supersedes the Swedish Standard SS-ISO 23611-3:2008, edition 1.

Förhållandet till övriga delar under samma huvudtitel - Utdrag ur Förord i ISO 23611-3:2007/ Relations to other parts under the same general title - Extract from the Foreword of ISO 23611-3:2007

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 free-living stages of terrestrial nematodes

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 23611-3

July 2011

ICS 13.080.30; 13.080.05

English Version

Soil quality - Sampling of soil invertebrates - Part 3: Sampling and soil extraction of enchytraeids (ISO 23611-3:2007)

Qualité du sol - Prélèvement des invertébrés du sol - Partie 3: Prélèvement et extraction des enchytréides (ISO 23611-3:2007)

Bodenbeschaffenheit - Probenahme von Wirbellosen im Boden - Teil 3: Probenahme und Bodenextraktion von Enchytraeen (ISO 23611-3:2007)

This European Standard was approved by CEN on 17 June 2011.

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Foreword

The text of ISO 23611-3:2007 has been prepared by Technical Committee ISO/TC 190 “Soil quality” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 23611-3:2011 by Technical Committee CEN/TC 345 “Characterization of soils” the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 2012, and conflicting national standards shall be withdrawn at the latest by January 2012.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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Endorsement notice

The text of ISO 23611-3:2007 has been approved by CEN as a EN ISO 23611-3:2011 without any modification.

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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 needed for the following purposes:

— biological classification of soils including soil quality assessment (e.g. References [21], [25], [27]);

— terrestrial bioindication and long-term monitoring (e.g. References [13], [26]);

— evaluation of the effects of chemicals on soil animals (References [15], [22]).

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 [5]). Their influence on soil functions like litter decomposition and nutrient cycling is well-known [14], [19]. Due to their number which is often very high (and to their population biomass), they are also important in many terrestrial food-webs [4]. 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 guidelines (e.g. ISO 10381-6 [29], ISO 14240-1 [37] and ISO 14240-2 [38])].

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Soil quality — Sampling of soil invertebrates —

Part 3:
Sampling and soil extraction of enchytraeids

1 Scope

This part of ISO 23611 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 part of ISO 23611 applies to all terrestrial biotopes in which enchytraeids occur. The sampling design of field studies in general is specified in ISO 10381-1. 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 micro-arthropods are specified in ISO 23611-1 and ISO 23611-2.

This part of ISO 23611 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 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.

2.1
enchytraeids
small soil-inhabiting worms (a few millimetres to several centimetres in length) belonging to the family Enchytraeidae, order Oligochaeta (class Clitellata, phylum Annelida)

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 corer (diameter usually 3 cm to 6 cm). 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 [11], [17], [20].) 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).

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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. Reference [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).

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).

5.12 Spring steel pincers (flat).

5.13 Eppendorf pipette, a soft steel forceps or a hooked needle.

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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 soil corer (5.1). The corer is carefully pressed into the soil. The depth depends on the 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 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 [7]). 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 in the water-saturated sample.

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 4 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 can be changed after 24 h or 48 h). 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 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 (0,5 mm) can be put in the mesh before the soil sample is put in [23].

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, an Eppendorf pipette 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 in 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, e.g. in 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 (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.