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Irish Standard  
I.S. EN ISO 23611-3:2011

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

## I.S. EN ISO 23611-3:2011

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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)

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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of an identical text or by endorsement, at the latest by January 2012, and conflicting national standards shall be withdrawn at the latest by January 2012.

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**Soil quality — Sampling of soil  
invertebrates —**

Part 3:  
**Sampling and soil 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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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.

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

ISO 23611-3 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 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<sup>[5]</sup>). Their influence on soil functions like litter decomposition and nutrient cycling is well-known<sup>[14], [19]</sup>. Due to their number which is often very high (and to their population biomass), they are also important in many terrestrial food-webs<sup>[4]</sup>. 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<sup>[29]</sup>, ISO 14240-1<sup>[37]</sup> and ISO 14240-2<sup>[38]</sup>)].



# 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)  $\alpha$ - and  $\beta$ -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  $\times$  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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