



SLOVENSKI STANDARD
oSIST prEN ISO 23611-5:2023
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Kakovost tal - Vzorčenje nevretenčarjev v tleh - 5. del: Vzorčenje in ekstrakcija velikih nevretenčarjev v tleh (ISO/DIS 23611-5:2023)

Soil quality - Sampling of soil invertebrates - Part 5: Sampling and extraction of soil macro-invertebrates (ISO/DIS 23611-5:2023)

Bodenbeschaffenheit - Probenahme von Wirbellosen im Boden - Teil 5: Probenahme und Extraktion von Makroinvertebraten (Großwirbellosen) im Boden (ISO/DIS 23611-5:2023)

Qualité du sol - Prélèvement des invertébrés du sol - Partie 5: Prélèvement et extraction des macro-invertébrés du sol (ISO/DIS 23611-5:2023)

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Soil quality — Sampling of soil invertebrates —

Part 5: Sampling and extraction of soil macro-invertebrates

*Qualité du sol — Prélèvement des invertébrés du sol —**Partie 5: Prélèvement et extraction des macro-invertébrés du sol*

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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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For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

ISO 23611-5 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*
- *Part 5: Sampling and extraction of soil macro-invertebrates*
- *Part 6: Guidance for the design of sampling programmes with soil invertebrates*

Introduction

This part of ISO 23611 was prepared in response to a need to standardize sampling and extraction methods for soil macro-invertebrates in several European (temperate) and tropical countries. These methods are needed for the following purposes:

- biological classification of soils, including soil quality assessment (e.g. References [14], [28] and [37]);
- terrestrial bio-indication and long-term monitoring (e.g. References [65], [74], [75] and [76]).

Data collected using standardized methods can be evaluated more accurately as it allows more reliable comparison between sites (e.g. polluted vs non-polluted sites, changes in land-use practices).

Soils of the world host an abundance of highly diverse macro-invertebrate communities. Their biology and ecology have been widely studied. Soil invertebrates are irreplaceable actors of soil formation and conservation in natural ecosystems. Their relevance to the soil system comes from their abundance and diversity, and also from their role in key biological processes. They are sensitive indicators of soil quality and recognized agents of its fertility (e.g. References [58] and [52]). Among the wide diversity of species, adaptive strategies and size ranges represented, one specific group, also called "soil ecosystem engineers", includes large invertebrates that actually determine the activities of other smaller organisms through the mechanical activities they produce in soil (e.g. References [18] and [46]).

Soil macro-invertebrates span a wide range of ecological functions in soil: decomposition of organic matter, through their own activity and by stimulating the soil's microbiological activity (e.g. References [2], [3] and [36]), predation that plays an important part in food webs (e.g. References [9], [51], [56], [59] and [63]), soil aggregation by the production of organo-mineral structures (e.g. nests, galleries, casts) that can last for days, months or years, and soil bioturbation (e.g. Reference [28]), etc. These characteristics, coupled with in-depth taxonomic knowledge, has enabled their use as study organisms in several research programmes dealing with the impacts of forest practices (e.g. References [11], [36], [47], [57], [60] and [70]) or crop management practices (e.g. References [8], [19], [27], [29], [30], [33], [38], [55] and [62]). These features make them suitable organisms for use as bio-indicators of changes in soil quality, especially with respect to land-use practices and pollution (e.g. References [21], [35], [45], [48], [49], [54], [60] and [74]).

The method proposed in this part of ISO 23611 covers the sampling of all soil macro-invertebrates. However, the sampling of earthworms is already covered in ISO 23611-1. This method is described in ISO 23611-1:2006, Annex C, as an alternative sampling method for earthworms.

Soil quality — Sampling of soil invertebrates —

Part 5:

Sampling and extraction of soil macro-invertebrates

1 Scope

This part of ISO 23611 specifies a method for sampling, extracting and preserving macro-invertebrates from soils, including the litter zone. The proposed method is a prerequisite for using these animals as bio-indicators (e.g. to assess the quality of a soil as a habitat for organisms). The main premise of this method is rapid assessment (completing the sampling of a plot in one or two days with only basic equipment and a small number of field assistants) in order to be able to address all the taxonomic groups of soil macro-invertebrates at the same time and in the same place. The Tropical Soil Biology and Fertility (TSBF) method has evolved and some modifications have been introduced in order to use it in temperate regions.

The sampling and extraction methods in this part of ISO 23611 are applicable to almost all types of soil, with the exception of soils in extreme climatic conditions (hard, frozen or flooded soils) and matrices other than soil, e.g. tree trunks, plants or lichens.

A sampling design is specified in ISO 23611-6.

NOTE 1 The method specified in this part of ISO 23611 is based on guidelines developed under the Tropical Soil Biology and Fertility Program (TSBF method)^[1].

NOTE 2 Basic information on the ecology of macro-invertebrates and their use can be found in the references listed in the Bibliography.

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 <https://www.electropedia.org/>

3.1

macro-invertebrates

soil organism whose longest dimension is greater than 10 mm

Note 1 to entry: See [Annex A](#) for further details.

EXAMPLE These include especially the following groups: Oligochaeta, Gastropoda, Chilopoda, Diplopoda, Isopoda, Arachnida, plus various insects: Coleoptera, Orthoptera, Hymenoptera, Hemiptera, Dermaptera, Lepidoptera (larvae) and Diptera (larvae).

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3.2

blotted mass

mass of individuals after preservation in formalin or ethanol (when the substance used for preservation has been absorbed by the tissues)

4 Principle

Soil macro-invertebrates are collected in the field using a metallic frame to delimit the soil surface of the sampling point. Macro-invertebrates present in litter and soil are picked up separately. In temperate regions, a reagent is used to extract macro-invertebrates from soil. The sampling is completed by hand-sorting. Animals are preserved and transported to the laboratory for further identifications (e.g. References [4], [5], [6], [7], [10], [12], [13], [16], [17], [22], [24], [25], [26], [31], [32], [34], [42], [43], [44], [50], [53], [64], [66], [67], [71], [72], [73] and [77]). Abundance values are usually recalculated relative to area (1 m²).

5 Reagents

5.1 Ethanol, (70 % volume fraction).

5.2 Formalin [formaldehyde solution], 4 % (volume fraction).

Both 70 % ethanol and 4 % formalin should be available for the preservation of specimens (4 % formalin is more suitable for taxa with soft body parts, which can be transferred to ethanol after about 4 d fixation).

5.3 Formalin, 0,2 % (volume fraction), prepared by diluting 25 ml of formalin (39 %) in 5 l of water, for soil macro-invertebrate extraction.

6 Apparatus

Use standard laboratory equipment and the following.

6.1 Petri dishes.

6.2 Stereo-microscope.

6.3 Plastic vials.

6.4 Entomological forceps.

6.5 Pencil, notebook, water-resistant marker, labels.

6.6 Tape measures.

6.7 Knife (cut glass).

6.8 Spade.

6.9 Plastic-weave produce sacks, for spreading on the ground.

6.10 Precision balance.

- 6.11 Large flat plastic trays** (500 mm × 400 mm × 100 mm), for sorting the soil and litter.
- 6.12 Trowel.**
- 6.13 Small plastic trays.**
- 6.14 Fine forceps (or entomological forceps), pipette, fine paint brushes.**
- 6.15 Sample vials**, in various sizes with secure alcohol-tight caps (plastic throw away or plastic/glass reusable vials)
- 6.16 Indian-ink pen** (waterproof).
- 6.17 Stiff card for labels, ranging compass.**
- 6.18 Large strong plastic bags** (sealable).
- 6.19 Table and plastic chairs**, for sorting.
- 6.20 Cover**, for protection from heavy rain.
- 6.21 Polyvinyl gloves**, to protect hands from formalin.
- 6.22 Metallic frame**, preferably 250 mm × 250 mm.
- Sample frame (250 mm × 250 mm × 50 mm) made of stainless steel and with sharpened edges to delimit the sampling point where animals are sampled from the litter layer and soil.
- <https://standards.iteh.ai/catalog/standards/sist/523c977e-8cf5-4fff-80be-cccce7c01cb5/osist-pren-iso-23611-5-2023>
- 6.23 Watering can.**
- 6.24 Pair of scissors**, to cut vegetation inside the frame.
- 6.25 Field balances.**

7 Field procedure

7.1 General

Sampling should take place when accessible biodiversity is thought to be largest. In temperate regions, it corresponds to spring or autumn, and in the tropics, it should take place towards the end of the rainy season.

When sampling soil invertebrates, it is strongly recommended that the site be physico-chemically characterized. In particular, pH, particle size distribution, C/N ratio, organic carbon content and water-holding capacity should be measured using ISO 10390, ISO 10694, ISO 11274, ISO 11277, ISO 11461, ISO 11465. Natural minerals present in the site soil should be also described.

7.2 Collecting macro-invertebrates from the litter zone

At each sampling point (= monolith) (previously defined according to sampling design rules), a litter sample is collected using a metallic frame (5.22). The metallic frame is pressed into the litter by hand. The litter inside the frame is removed and checked manually in the field using a large tray (5.11). Litter invertebrates are preserved in 4 % formalin (4.2).