SLOVENSKI STANDARD
oSIST prEN ISO 23611-2:2023

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Kakovost tal - Vzorčenje nevretenčarjev v tleh - 2. del: Vzorčenje in ekstrakcija mikročlenonožcev: skakači (Collembola) in pršice (Acarina) (ISO/DIS 23611-2:2023)

Soil quality - Sampling of soil invertebrates - Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina) (ISO/DIS 23611-2:2023)

Bodenbeschaffenheit - Probenahme von Wirbellosen im Boden - Teil 2: Probenahme und Extraktion von Mikroarthropoden (Collembolen und Milben) (ISO/DIS 23611-2:2023)

Qualité du sol - Prélèvement des invertébrés du sol - Partie 2: Prélèvement et extraction des micro-arthropodes (Collembola et Acarina) (ISO/DIS 23611-2:2023)

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Soil quality — Sampling of soil invertebrates —

Part 2:
Sampling and extraction of micro-arthropods (Collembola and Acarina)

Qualité du sol — Prélèvement des invertébrés du sol —

Partie 2: Prélèvement et extraction des micro-arthropodes (Collembola et Acarina)

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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 23611-2 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 free-living stages of terrestrial nematodes*

Introduction

This part of ISO 23611 has been drawn up since there is a growing need for the standardization of sampling and extraction methods of soil micro-arthropods. These methods are needed for the following purposes:

— biological classification of soils including soil quality assessment (e.g. References [19], [24], [27], [30], [36], [40], [41]);

— terrestrial bioindication and long-term monitoring (e.g. References [3], [12], [14], [19], [31], [34], [37]).

Data collected by standardized methods can be more accurately evaluated allowing more reliable comparisons between sites (e.g. polluted versus non-polluted sites, changes in land-use practices).

From the several micro-arthropod groups, Collembola and Acarina are the most studied in soil ecology. Their relevance for the soil system comes from their high abundance and diversity, and also from their role in key biological processes. Collembola and Oribatid mites act mainly as catalysts in organic matter decomposition[6],[21], whereas predacious mites may act as webmasters in soil food webs[21],[26]. These characteristics, allied to a widespread taxonomic knowledge, allowed their use as study organisms in several research programmes dealing with the impacts of forest practices (e.g. References [8], [16], [17], [18], [22], [23], [24], [28], [29], [32], [33], [35], [42]) or crop management practices (e.g. [2], [7], [10], [13], [20], [25], [43], [44]). These features make them suitable organisms to be used as bio-indicators of changes in soil quality, especially due to land-use practices and pollution[38].

Soil quality — Sampling of soil invertebrates —

Part 2:
Sampling and extraction of micro-arthropods (Collembola and Acarina)

1 Scope

This part of ISO 23611 specifies a method for sampling, extracting and preserving collembolans and mites from field soils as a prerequisite for using these animals as bio-indicators (e.g. to assess the quality of a soil as a habitat for organisms).

Basic information on the ecology of micro-arthropods and their use can be found in the references listed in the Bibliography.

The sampling and extraction methods of this part of ISO 23611 are applicable to almost all types of soils. Exceptions may be soils from extreme climatic conditions (hard, frozen or flooded soils) and other matrices than soil, e.g. tree trunks, plants or lichens. For the sampling design of field studies in general, see ISO 18400-104.

Methods for some other soil organism groups, such as earthworms, are covered in other parts of ISO 23611.

This part of ISO 23611 does not cover the pedological characterization of the site, which is highly recommendable when sampling soil invertebrates. 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 https://www.electropedia.org/

3.1
micro-arthropods
group which is defined by its small size (range size from 100 µm to a few millimetres) making up a significant part of the below-ground food web in many terrestrial ecosystems

Note 1 to entry: This group is mainly composed by mites (Acarina), springtails (Collembola), Protura, Diplura, garden centipedes (Symphyla), Pauropoda, small centipedes and millipedes, small arachnids (spiders and pseudoscorpions), and insects and their larvae from several orders (Hymenoptera, Diptera, Coleoptera, etc.).

4 Principle

Soil samples are collected in the field using a split corer. Soil cores are placed in plastic tubes (or plastic bags) and transported to the laboratory. Afterwards, Collembola and Acarina are rapidly (within a few days) extracted by behavioural methods, using a MacFadyen apparatus, and preserved for future identifications[12],[34]. In addition, preparation techniques are also described. Finally, abundance values can be recalculated related to area (usually 1 m^2), volume or weight (usually 1 kg).

NOTE Alternative methods for extraction can be used under special circumstances. Flotation methods (e.g. the heptane flotation method) can be used in clay or loamy soils and a Kempson extractor (5.19) is advisable in the case litter is sampled[34].

5 Test materials

5.1 Biological material

Collembola (springtails) are small wingless hexapods (from 150 µm up to 9 mm length), having a distinctive head with a pair of antennae, without true compound eyes, with six abdominal segments and three pre-genital appendages in the abdomen. In the first segment, there is the ventral tube (or collophore) that is used for adhering to smooth surfaces. The name Collembola comes from this structure (from Greek *colla* = glue and *embolon* = bar). In the third segment, there is the *tenaculum*, which holds the jumping apparatus on its normal position. This jumping appendage, the *furcula* (or spring), is located in the fourth segment, when present. Springtails live in litter and soil, and have very distinctive life forms. They belong to the class Collembola and can be separated into 33 families[4].

Soil mites are small chelicerate arthropods related to spiders (length from 150 µm up to < 5 mm), living in soil and litter, and also presenting very distinctive life forms. They belong to the class Arachnida, subclass Acarina, and can be separated into four groups: Cryptostigmata (Oribatida), Mesostigmata (Gamasida), Prostigmata (Trombidiformes) and Astigmata.

NOTE Some hints for the taxonomy of springtails and mites are given in Annex A.

5.2 Reagents

Unless otherwise specified, use only reagents of good quality and distilled water.

5.2.1 Propan-2-ol, 80 % (volume fraction).

5.2.2 Formalin [formaldehyde solution 40 % (volume fraction)].

5.2.3 Acetic acid.

5.2.4 Phenol, C_6H_5OH, crystalline (carbolic acid).

5.2.5 Hydrogen chloride, *c*(HCl) from 8 mol/l to 10 mol/l.

5.2.6 2,2,2-Trichloro-1,1-ethanediol (chloral hydrate).

5.2.7 1,2,3-Trihydroxypropane (glycerine).

5.2.8 von Törne fixative, used to preserve the extracted animals and composed by Propan-2-ol (80 %), formalin (40 %) and glacial acetic acid (a volume fraction 10:0,3:0,03).

5.2.9 Nesbitt clearing medium, used to clear mite specimens composed of chloral hydrate (80 g), distilled water (50 ml) and concentrated hydrogen chloride (5 ml).

5.2.10 Lactophenol solution, used to clear mite specimens composed of lactic acid (10 ml), crystals of phenol (3,6 g) and distilled water (5 ml).

5.2.11 2-Hydroxypropanoic acid (lactic acid), to clear and observe micro-arthropod specimens, especially oribatid mites under the microscope.

5.2.12 Ethanol, 70 % to 75 % (volume fraction), used for fixation and preservation (in this case, also in combination with glycerine, 10:1).

5.2.13 Hoyer's medium, used to mount Collembola specimens composed of distilled water (50 ml), gum-arabic (30 g), chloral hydrate (200 g) and glycerine (20 ml).

5.2.14 DNA extraction buffer (SNET buffer solution), used to clear collembolans.

5.2.15 Protease K solution, used to clear collembolans.

5.2.16 Ethanol 35 % (volume fraction), used for preservation of the specimens.

5.2.17 Formol 3 %, used for preservation of the specimens.

5.2.18 Marc André 2 medium, to clear and provide the best optical properties to the specimens for identification.

6 Apparatus

Use standard laboratory equipment and the following.

6.1 Measuring tape.

6.2 Collecting flasks.

6.3 Wash bottle.

6.4 Forceps, **pipette**, **fine painting brush**, **fine needles**.

6.5 Petri dishes.

6.6 Stereomicroscope.

6.7 Microscope, with phase or interference contrast is preferable.

6.8 Microscopic slides, with excavated area in the centre, and **lamellae**.

6.9 Electrical heating plate.

6.10 Plastic vials.

6.11 Ceramic heating elements.

6.12 Pencil, **notebook**, **water resistant marker**, **labels**.