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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.

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 23611-2 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 [31], [32], [35], [41], [45], [46]);

— terrestrial bioindication and long-term monitoring (e.g. References [1], [7], [17], [40], [42]).

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 [4], [20], whereas predacious mites may act as webmasters in soil food webs [9]. 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 [12], [13], [14], [15], [18], [19], [21], [22], [23], [25], [26], [27], [28], [29], [30], [31], [33], [34], [37], [38], [39]) or crop management practices (e.g. [6], [11], [16], [24]). 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 [43].

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 10381-1.

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 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
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 This group is mainly composed by mites (Acarina), springtails (Collembola), Protura, Diplura, garden centipedes (Symphyla), Pauropoda, small centipedes and millipedes, and insects and their larvae from several orders (Diptera, Coleoptera, etc.).

3 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 Acarida are rapidly (within a few days) extracted by behavioural methods, using a MacFadyen apparatus, and preserved for future identifications [7], [40]. 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 is advisable in the case litter is sampled [40].

4 Test materials

4.1 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*, that holds the jumping apparatus on its normal position. This jumping appendage, the *furcula* (or spring), when it exists, is located in the fourth segment. Springtails live in litter and soil, and have very distinctive life forms. They belong to the class Collembola, and can be separated into 18 families [17].

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 Acarida, 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.

4.2 Reagents

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

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

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

4.2.3 Acetic acid.

4.2.4 Phenol, C_6H_5OH, crystalline (carbolic acid).

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

4.2.6 2,2,2-Trichloro-1,1-ethanediol (chloral hydrate).

4.2.7 1,2,3-Trihydroxypropane (glycerine).

4.2.8 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).

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

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

4.2.11 2-Hydroxypropanoic acid (lactic acid), to clear and observe micro-arthropod specimens, especially oribatid mites under the microscope.

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

4.2.13 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 Apparatus

Use standard laboratory equipment and the following.

5.1 Measuring tape.

5.2 Collecting flasks.

5.3 Wash bottle.

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

5.5 Petri dishes.

5.6 Stereomicroscope.

5.7 Microscope, with phase or interference contrast is preferable.

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

5.9 Electrical heating plate.

5.10 Plastic vials.

5.11 Ceramic heating elements.

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

5.13 Split corer

Sampling device made of stainless steel or aluminium (40 cm long and e.g. 5,6 cm diameter may be used; the size and diameter should not differ considerably from these numbers in order to maintain comparable conditions), used to collect soil cores (samples). It can be composed of two independent parts that fit together along the corer main axis or it can consist of one tube. On the top, it has a handle and on the bottom, a cutting edge.

5.14 MacFadyen apparatus

High-gradient (multiple) device used to extract micro-arthropods from soil samples. The principle is to create an artificial temperature gradient between the canister where the sample is placed (hot) and the collecting device below (cold), inducing a negative thermotactic (at the same time a positive hygrotactic, negative phototactic and positive skototactic) behaviour on the animals that, by this way, leave the soil sample.

5.15 Plastic tubes, with caps (5 cm diameter, 5 cm long), or **plastic bags**, for storing the soil samples.

5.16 Kempson extractor, in the case litter is sampled.

5.17 Sample frame, 25 cm × 25 cm × 15 cm, made of stainless steel and with sharpened edges, to sample animals from the litter layer.

NOTE For details concerning the equipment in 5.13 to 5.17, see References [7] and [40].

6 Procedure

6.1 Collecting the soil samples

At each sampling point (previously defined according to sampling design rules), a soil sample is collected using a split corer (5.13); for flooded soils the same corer may be employed, but an auger tip should be present to retain the soil after extraction.

NOTE In addition to the general characterization of the site (see Clause 1), it is useful to determine the actual moisture of the soil to be sampled.

After the sample is taken, the corer is opened and the soil core is separated into litter layer (including the humus horizon) and the upper 10 cm of the mineral soil. Generally 5 cm layers are used for the upper part of the mineral horizon, but if a finer analysis is required, thinner layers can be defined. The depth of the litter layer should be registered. After this procedure, each layer is conditioned in plastic tubes; these are sealed with caps, labelled, and stored for transportation to the laboratory. Plastic bags can be used as substitutes of the plastic tubes (5.15), but special care shall be taken during conditioning to avoid disturbing the core structure and compaction of the soil material, that may lead to the death of animals. The time lapse between sampling and extraction should not exceed a few days, in order to avoid undesirable side effects due to confinement and shifts in micro populations.

If sampling of animals is restricted to the litter layer, a sample frame (5.17) is used instead. The frame is pressed into the litter by hand. Directly afterwards, the litter inside the frame is collected and the litter samples are placed in plastic bags (5.15), labelled and stored.

6.2 Extracting Collembola and Acarina from soil samples

In the laboratory, animals are extracted by behavioural methods, e.g. using a MacFadyen high-gradient extractor (5.14). Each sample core is placed inverted into the canister having a plastic or metal net (2 mm mesh size) on the bottom. This is connected to a funnel attached to a collecting flask (5.2) with 25 ml of “von Törne-fixative” (4.2.8).

Alternatively, a saturated solution of picric acid, a 50 % ethylene glycol solution (plus some drops of a detergent) or even 75 % ethanol (4.2.12) may be used as fixative.

A temperature gradient is created between the upper part (where the samples are) and the lower part of the system (where the collecting flasks are placed). Heat can be provided by ceramic heating elements (5.11), giving approximately 10 W per sample. The collecting flasks are immersed in a cooling water bath. In some commercial apparatus, the temperature gradient is obtained by circulating heated air in the canister area and cooled air on the collecting area.

The temperature difference between the upper and lower parts should be around 30 °C to 35 °C, with the upper part being heated at 45 °C to 50 °C and the lower part being cooled usually at 10 °C (maximum field temperature). Special care shall be taken in order to avoid a fast increase in temperature in the upper part, which may cause the rapid desiccation of the sample and the death of the animals. Therefore, it is recommended to have a gradual increase of the temperature of the upper part, starting with approximately 5 °C above field temperature during the first three days, and intensifying the gradient for the next six to seven days.

The extraction procedure takes nine to ten days. Afterwards, animal samples are labelled and ready to be stored until processing (sorting and identification). Extraction should preferably start as soon as possible (i.e. the day of sampling). In case storage is necessary, the soil samples should be kept at 4 °C.

NOTE 1 The method described here is only efficient for active live stages, with an average extraction efficiency of 75 % to 80 % [3], [41]. Eggs, other quiescent stages and animals enclosed in plant debris are not extracted by this method; alternatively, the heptane flotation method [44] can be used (see Annex B).

