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Soil quality — Sampling of soil invertebrates~~—~~
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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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Contents

Foreword

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This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*.

This second edition cancels and replaces the first edition (ISO 23611-2:2006), which has been technically revised.

The main changes are as follows:

— ~~An~~an additional Note was added in Clause 7.3.2.1~~Clause 7.3.2.1~~ with the description of an alternative method to the classic pre-heating techniques for ~~specimens~~specimen preparation for Collembola taxonomic identification~~.~~;

— ~~The~~the bibliographic references list was revised and updated ~~in the entire document.~~.

A list of all parts in the ISO 23611 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document was prepared in response to 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~~[19], [24], [27], [30], [36], [40], [41]);~~]);

— ~~—~~terrestrial bioindication and long-term monitoring (e.g. References [3], [12], [14], [19], [31], [34], [37~~[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[6],[21],~~] whereas predacious mites can act as webmasters in soil food webs.~~[21],[26[21],[26],~~] These characteristics, allied to a widespread taxonomic knowledge, allow 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~~[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~~. [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[38],~~].

For the sampling design of field studies in general, see ISO 18400-104~~[45 [45]~~] for general guidance on the development of site investigation strategies and detailed guidance on the development of sampling strategies.

Methods for other soil organism groups, such as earthworms, ~~Enchytraeids~~enchytraeids, nematodes and macro-invertebrates are covered in ISO 23611-1~~[52],~~ ISO 23611-3~~[53],~~ ISO 23611-4~~[54]~~ and ISO 23611-5~~[55],~~ respectively.

Soil quality — Sampling of soil invertebrates —

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

1 Scope

This document 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).

The sampling and extraction methods of this document are applicable to almost all types of soils. Exceptions ~~may~~can be soils from extreme climatic conditions (hard, frozen or flooded soils) and other matrices than soil, e.g. tree trunks, plants or lichens.

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 terminology 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/

~~Micro~~3.1
micro-arthropod
~~Group~~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][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 (6.18~~(6.18) is advisable~~) can be used in the case litter is sampled[34][34].].

5 Test materials

5.1 Biological material

Collembola (springtails) are small wingless hexapods (from 150 µm up to 9 mm in 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 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**.

6.13 Split corer

Sampling device made of stainless steel or aluminium (40 cm long and e.g. 5,6 cm diameter may be used; the length 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.

6.14 Glass vials.

6.15 Drying oven.

6.16 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.

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

6.18 Kempson extractor, in the case litter is sampled.

6.19 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 6.13~~6.13,~~ and 6.16~~6.16~~ to 6.19~~6.29,~~ see References [12~~[12]~~] and [34~~[34].~~].

7 Procedure

7.1 Collecting the soil samples

At each sampling point (previously defined according to sampling design rules), a soil sample is collected using a split corer (6.13~~(6.13);~~); 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, it is useful to determine the actual moisture of the soil to be sampled.

After the sample is taken, the corer is opened (a picture of the soil core profile can complement the site characterization) 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 (6.17~~(6.17),~~), but special care shall be taken during conditioning to avoid disturbing the core structure and compaction of the soil material, that can lead to the death of animals. The time lapse between sampling and extraction should be recorded and should not exceed 5 days (if the samples remain at 20~~ ± 2ºC~~ °C ± 2 °C and the soil is kept moist), 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 (6.19~~(6.19)~~) 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 (6.17~~(6.17),~~), labelled, and stored.

When sampling in soil, the site should 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[46 ~~[46],~~] ISO 10694[47 ~~[47],~~] ISO 11274[48 ~~[48],~~] ISO 11277[49 ~~[49],~~] ISO 11461[50 ~~[50],~~] ISO 11465[51 ~~[51].~~].

7.2 Extracting Collembola and Acarina from soil samples

In the laboratory, animals are extracted by behavioural methods, e.g. using a MacFadyen high-gradient extractor (6.16~~(6.16).~~). 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 (6.2~~(6.2)~~) with 25 ml of "von Törne-fixative" (5.2.8~~(5.2.8).~~).

Alternatively, a saturated solution of picric acid, a 50 % ethylene glycol solution (plus some drops of a detergent) or even 75 % ethanol (5.2.12~~(5.2.12)~~) may be used as fixative.

A temperature gradient is created between the upper part (where the samples are placed) and the lower part of the system (where the collecting flasks are placed). Heat can be provided by ceramic heating elements (6.11~~(6.11),~~), giving approximately 10 W per sample. The collecting flasks are immersed in a cooling