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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

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This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This second edition cancels and replaces the first edition (ISO 23611-5:2011), which has been technically revised.

The main changes are as follows:

— Two informative Annexes were added at the end of the document. Annex B describes the procedures to be adopted when sampling macro-fauna using pitfall traps and Annex C presents a monitoring example with pitfall traps.

— 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 need to standardize sampling and extraction methods for soil macro-invertebrates globally. 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 they allow 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 actors 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 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, soil bioturbation (e.g. Reference [28]), etc. These characteristics, coupled with in-depth taxonomic knowledge, have 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 document covers the sampling of all soil macro-invertebrates. However, the sampling of earthworms is already covered in ISO 23611-1. This alternative sampling method for earthworms is described in ISO 23611-1:2018, Annex C.

The method proposed in this document is a prerequisite for using macro-invertebrates 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.

A sampling design is specified in ISO 23611-6.

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

Soil quality — Sampling of soil invertebrates —

Part 5:
Sampling and extraction of soil macro-invertebrates

1 Scope

This document specifies a method for sampling, extracting and preserving macro-invertebrates from soils, including the litter zone.

The sampling and extraction methods in this document 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.

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/

3.1
macro-invertebrate
soil organism whose longest dimension is greater than 10 mm

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).

Note 1 to entry: See Annex A for further details.

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 identification (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 m^2).

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 Chemical protection gloves, suitable for working with 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.

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, 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, ISO 10694, ISO 11274, ISO 11277, ISO 11461, ISO 11465. Natural minerals present in the site soil should also be 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 (6.22). 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 (6.11). Litter invertebrates are preserved in 4 % formalin (5.2).

7.3 Collecting macro-invertebrates from soil

7.3.1 General

In temperate countries, the extraction of soil macro-invertebrates is carried out in two steps (see 7.3.2.1 and 7.3.2.2), while in tropical countries only the second step shall be performed (see 7.3.3). In both cases, extraction of macro-invertebrates may be complemented by the use of pitfall traps (see Annexes B and C for further details).

7.3.2 Temperate regions

7.3.2.1 Formalin extraction

The soil surface delimited by the metallic frame (6.22) is sprayed with 0,2 % formalin (5.3) using a watering can (6.23). Two applications of 1,5 l of formalin are performed at intervals of about 10 min. Soil invertebrates coming up to the surface are collected and preserved in vials (6.3) containing formalin (5.2).

7.3.2.2 Hand-sorting of "passive" macro-invertebrates

At the end of the formalin extraction, the metallic frame (6.22) is removed and the upper 150 mm of soil is excavated within the frame area (250 mm × 250 mm). The excavated soil is placed in a plastic bag (6.18) that can be closed with a cover to prevent animals from escaping from the soil sample.

Appropriate sub-samples of soil are taken from the container and spread on a large tray (6.11). Macro-invertebrates are collected and preserved in vials (6.3) with formalin (5.2). When hand-sorting is finished, the excavated soil is replaced to avoid creating holes on the sampling site.

7.3.3 Tropical regions

In tropical countries, soil macro-invertebrates are sampled using a 250 mm × 250 mm × 300 mm deep soil monolith. The monolith is isolated by cutting with a spade (6.8) a few centimetres outside the quadrate (metallic frame) and then digging a 20 mm wide by 300 mm deep trench around it. This facilitates cutting of the sample into horizontal strata and collecting animals escaping from the block.

The delimited block is divided into three layers, 0 mm to 100 mm, 100 mm to 200 mm and 200 mm to 300 mm; and the soil and litter material is hand-sorted in trays (6.11). Since formalin is not applied in tropical regions, the sampling depth should be doubled in order to be sure to collect endogeic and anecic species of earthworms.

For social insects, special measures should be considered that take account of their high abundance and marked patchiness; a nest can contain millions of individuals, of which none are sampled by a short transect, and the contribution of the species concerned to a macrofaunal assemblage can thus be completely missed. On the other hand, a highly populated nest sampled directly by a monolith can lead to a large overestimation of the overall numerical or biomass density. In general, the TSBF transect should be placed to avoid direct contact with termite and ant nests. For discussions, see References [35] and [36]. The protocol for a 100 m × 2 m transect designed to assess termite biodiversity (and feeding group representation) is given in Reference [48]. In suitable circumstances, this protocol can also be deployed in parallel with the TSBF transect.

NOTE Besides the general characterization of the site, it is useful to determine the actual moisture of the soil to be sampled.

8 Laboratory procedure

8.1 Treatment of collected samples

In the laboratory, samples are cleaned with either distilled or tap water in a Petri dish with the help of a brush or placing the organisms on a 0,5 mm to 1 mm sieve under the tap. Afterwards, the animals are placed in new vials (6.15) with ethanol (70 % volume fraction) (5.1). Organisms with soft body parts are kept in formalin for at least 4 d, or forever if possible.

For taxonomic identification, specimens are placed on petri dishes (6.1) and observed under the stereo-microscope (6.2). A practical way to identify macro-invertebrates is to group them into orders first. Each order is then identified into families and each family into species using taxonomy keys (examples of taxonomy keys are the References [4], [5], [6], [7], [10], [12], [13], [17], [22], [24], [25], [26], [31], [32], [34], [42], [43], [44], [53], [66], [67], [72], [73] and [77]).

Ideally, taxonomic determination should be based on the species level. If identification of species levels fails due to time constraints, taxonomic expertise or missing taxonomic keys, e.g. mainly in tropical regions, sorting to genus (and some higher taxonomic units) represents a good compromise between the morphospecies and ordinal level approaches, especially as this allows most specimens to be assigned to a functional group.

WARNING — Appropriate precautions (i.e. gloves, mask) should be taken when dealing with formalin to avoid danger from inhalation or skin exposure. According to the Material Safety Data Sheet for formaldehyde 37 % solution published by producing companies, the compound is a skin sensitizer