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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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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 23611-5 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 [21], [32] and [41]);

— terrestrial bio-indication and long-term monitoring (e.g. References [71], [79], [80] and [81]).

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 [63] and [56]). 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 [24] and [49]).

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 [8], [10] and [40]), predation that plays an important part in food webs (e.g. References [16], [55], [61], [64] and [68]), 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 [32]), 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 [18], [40], [50], [62], [65] and [75]) or crop management practices (e.g. References [15], [25], [31], [33], [34], [37], [42], [60] and [66]). 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 [26], [39], [48], [52], [53], [59], [65] and [79]).

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)[7].

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

2 Terms and definitions

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

2.1
macro-invertebrates
soil organism whose longest dimension is greater than 10 mm

NOTE 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).

2.2
blotted mass
mass of individuals after preservation in formalin or ethanol (when the substance used for preservation has been absorbed by the tissues)

3 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 [11], [12], [13], [14], [17], [19], [20], [22], [23], [27], [28], [29], [30], [35] ,[36], ,[38], [45], [46], [47], [54], [57], [70], [72], [73], [76], [77], [78] and [84]). Abundance values are usually recalculated relative to area (1 m^2).

4 Reagents

4.1 Ethanol, (70 % volume fraction).

4.2 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).

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

5 Apparatus

Use standard laboratory equipment and the following.

5.1 Petri dishes.

5.2 Stereo-microscope.

5.3 Plastic vials.

5.4 Entomological forceps.

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

5.6 Tape measures.

5.7 Knife (cut glass).

5.8 Spade.

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

5.10 Precision balance.

5.11 Large flat plastic trays (500 mm × 400 mm × 100 mm), for sorting the soil and litter.

5.12 Trowel.

5.13 Small plastic trays.

5.14 Fine forceps (or entomological forceps), pipette, fine paint brushes.

5.15 Sample vials, in various sizes with secure alcohol-tight caps.

5.16 Indian-ink pen (waterproof).

5.17 Stiff card for labels, ranging compass.

5.18 Large strong plastic bags (sealable).

5.19 Table and plastic chairs, for sorting.

5.20 Cover, for protection from heavy rain.

5.21 Polyvinyl gloves, to protect hands from formalin.

5.22 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.

5.23 Watering can.

5.24 Pair of scissors, to cut vegetation inside the frame.

5.25 Field balances.

6 Field procedure

6.1 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.

6.2 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).

6.3 Collecting macro-invertebrates from soil

6.3.1 General

In temperate countries, the extraction of soil macro-invertebrates is carried out in two steps (see 6.3.2.1 and 6.3.2.2), while in tropical countries only the second step shall be performed (see 6.3.3).

6.3.2 Temperate regions

6.3.2.1 Formalin extraction

The soil surface delimited by the metallic frame (5.22) is sprayed with 0,2 % formalin (4.3) using a watering can (5.23). 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 (5.3) containing formalin (4.2).

6.3.2.2 Hand-sorting of “passive” macro-invertebrates

At the end of the formalin extraction, the metallic frame (5.22) 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 (5.18) 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 (5.11). Macro-invertebrates are collected and preserved in vials (5.3) with formalin (4.2). When hand-sorting is finished, the excavated soil is replaced to avoid creating holes on the sampling site.

6.3.3 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 (5.8) 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 (5.11). Since formalin is not applied in tropical regions, the sampling depth shall be doubled in order to be sure to collect endogenic species of earthworms.

For social insects, it is recommended that special measures 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 so as to avoid direct contact with termite and ant nests. For discussions, see References [39] and [40]. The protocol for a 100 m × 2 m transect designed to assess termite biodiversity (and feeding group representation) is given in Reference [52]. 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.

7 Laboratory procedure

7.1 Treatment of collected samples

In the laboratory, samples are cleaned and animals are placed in new vials (5.15) with ethanol (70 % volume fraction) (4.1). 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 (5.1) and observed under the stereo-microscope (5.2). A practical way to identify macro-invertebrates is to group them first into orders. Each order is then identified into families and each family into species using taxonomy keys (examples of taxonomy keys are given in the Bibliography (see Rerences [11], [12], [13], [14], [17], [19], [20], [23], [27], [28], [29], [30], [35], [36], [38], [45], [46], [47], [57], [72], [73], [77], [78] and [84]).

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) shall 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 and is considered to be carcinogenic (humans: limited evidence; animals: sufficient evidence). It is legally notified in industrialized countries for scientific use.

7.2 Preservation of specimens

From any mixed soil sample of macrofauna, the following steps should be followed in order to obtain standardized preserved specimens.

a) If the animal has no soft body parts, the organisms should be preserved in 70 % ethanol (commercial ethanol should be diluted).

b) If the animal has soft body parts, the organism should be fixed in 4 % formalin and should, if possible, be preserved in the same solution. Alternatively, 80 % ethanol could be used (if the organism has been fixed during at least 4 d with 4 % formalin).

c) In all cases, samples should be stored separately in different vials, according to the smallest unit of analysis (i.e. a monolith if the data is compared at that level).

d) Every vial should be labelled without using code numbers and should at least be written using permanent ink, like Indian or Chinese ink, and using sturdy paper like goatskin parchment. Every label should contain the following information:

   — country;

   — region;

   — locality;

   — collector's name;

   — date of collection.

e) For storing specimens:

   — use vials (or glass tubes) that are not degraded by the ethanol or formalin, with screw caps;

   — monitor levels of ethanol and formalin in order to keep them constant;

   — store vials away from direct sunlight;

   — change the preserving solution of each vial once every five years.

7.3 Biomass determination

Determination of biomass is performed using the preserved material. The animal's surface should be gently dried with filter paper, then weighed using a precision balance (0,001 g).

It is virtually impossible to keep invertebrates alive after their capture in order to measure fresh masses. In most cases, invertebrates are conserved in 70 % (volume fraction) ethanol or 4 % (volume fraction) formalin. The latter is recommended for earthworms that shall at least be fixed in formalin before being kept in 70 % ethanol. Preservation always involves a decrease in mass, as body water is extracted by osmotic forces. The amount lost can vary between 15 % and 40 %, depending on the water content of the animal and its physiological state. Since most studies only aim to compare different sites and/or situations, mass loss is not likely to distort the result. If accurate fresh mass data are necessary, it is easy to keep an aliquot of each group and compare the mass, alive and fixed, a few days after fixation.

8 Assessment of results

The following measurement end points can be used for the bioclassification of a soil, including bio-indication or biomonitoring (e.g. anthropogenic stress-like chemicals or land-use changes):

— abundance (number of individuals per area);

— biomass;

— number of species or other taxonomically or ecologically defined groups;

— diversity indices (alpha, beta and gamma diversity).

Firstly, the number of individuals (total number by species or group) is counted and expressed as individuals per sample. Secondly, the total abundance of individuals is multiplied by a factor (16) to obtain the number of individuals per square metre.

Fresh mass measured in the field is the ideal way to calculate biomass. Failing this, the use of blotted mass, after preservation, is acceptable. Other methods are reported in the literature, for example fresh mass after blotting, dry mass at 60 °C overnight, drying to constant mass at higher temperatures, degutted fresh mass, degutted dry mass, fresh mass × a constant (for assumed water content) and head width (referenced to a calibration curve). However, these have less biological meaning than fresh mass.

9 Test report

The test report shall include at least the following information:

a) a reference to this part of ISO 23611, i.e. ISO 23611-5:2011);

b) a full description of the study design and procedures;

c) characterization of the study site (especially soil properties);

d) sampling method;

e) description of the sampling conditions, including date and duration of sampling in the field and weather parameters like air temperature and humidity, rain or snow, etc.;

f) details of the extraction procedure of the biological material;

g) values recalculated to 1 m^2 or another standard size, if necessary;

h) a summary of the results obtained;

i) a discussion of the results;

j) all information, including all measured raw data and all problems which might have occurred or developed during all phases of the study.

Annex A
(normative)

Background information

Soil macro-invertebrates can also be defined as organisms belonging to taxa of which over 90 % of specimens are visible to the naked eye. Soil macro-invertebrates comprise the following groups: Oligochaeta (Annelida), Gastropoda (snails and slugs), Coleoptera (larvae and adults), Isoptera, Diplopoda, Chilopoda, Hymenoptera, Arachnida, Dyctioptera, Orthoptera, Hemiptera, Dermaptera, Isopoda, Lepidoptera (larvae) and Diptera (larvae). It specifically excludes groups with a relatively small number of specimens visible to the naked eye such as Nematoda (e.g. *Mermithidae*), Enchytraeidae, Collembola, Acarina, Symphyla, Pauropoda and Diplura. Core taxonomic units should be adopted as standard units for macrofaunal sampling. The choice of 17 main taxa was made during the IBOY Workshop using the MDB (Macrofauna Data Base) containing information about 32 countries and almost 1 000 sampled sites. The 17 taxa, Oligochaeta (order *opisthopora*), Coleoptera (larvae and adults), Isoptera, Diplopoda, Chilopoda, Formicidae, Gastropoda, Araneae, Blattoidea, Orthoptera, Dermaptera, Isopoda, Hemiptera, Lepidoptera larvae, Diptera (larvae and adults) and residues (insects and non-insects), correspond to the most important soil macro-invertebrates in terms of abundance and biomass.

Choice of a 250 mm × 250 mm × 300 mm monolith size is based on extensive, although largely empirical, experience. First used by Zajonc (1956), it has been proposed as a standard for the Tropical Soil Biology and Fertility Program[7][59]. This monolith size is the same for both tropical and temperate soils. The aim was to propose a method that was not excessively time-consuming, but which would provide an accurate assessment of the composition and structure of soil macro-invertebrate communities. The method has been extremely successful and has become a standard used in several hundred sites. Although studies aimed at specific groups, especially termites, earthworms or ants, prefer different sample sizes, the size proposed represents a very good compromise that allows a reasonable number of replicates to be made and the representation of most orders in one single sample. Larger samples are excessively time-consuming and do not allow enough replicates to be made. In most cases, a group of four well-trained persons can sort out 10 samples a day. Unpublished field studies have shown that 15 to 20 samples are necessary in a single site to reduce variance to a reasonably low proportion of mean (<20 %). However, a comparison of sites with different plant cover or soils and/or which have be subjected to different management options exposes significant differences using as little as five samples, provided adapted statistical treatment (often multivariate analyses) is used.

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[4] ISO 11277, *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation*

[5] ISO 11461, *Soil quality — Determination of soil water content as a volume fraction using coring sleeves — Gravimetric method*

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