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Soil quality — Sampling of soil invertebrates —

Part 6:
Guidance for the design of sampling programmes with soil invertebrates

Qualité du sol — Prélèvement des invertébrés du sol —

Partie 6: Lignes directrices pour la conception de programmes d'échantillonnage des invertébrés du sol

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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 23611-6 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-arthopods (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

The biodiversity of soil fauna is tremendous. Soil harbours species-rich communities, which regulate ecosystem processes such as organic matter decomposition, nutrient flows or soil fertility in general, References [40], [45]. All terrestrial animal phyla can be found in soils, Reference [16]. In addition to thousands of bacterial and fungal “species“, more than 1 000 species of invertebrates in abundances of up to 1,5 million individuals can be found within a square metre of soil, References [3], [5]. This diversity can only be reliably estimated by investigation of the soil community itself, since other parameters like climate are not or only weakly correlated with species richness, Reference [24].

The composition of this community, as well as the abundance and biomass of the individual species and groups is a valuable source of information, since they integrate various abiotic and biotic effects such as soil properties and conditions, climate, competition or biogeographical influences, Reference [68]. For this reason, the evaluation of the biodiversity of soil invertebrate communities becomes more and more important for the classification and assessment of biological soil quality, Reference [51]. However, this work is only possible if data collection (i.e. sampling of the soil fauna) is carried out according to standardized methods. For this reason, a number of ISO guidelines have been prepared covering the sampling of the most important soil organism groups.

In the individual parts of ISO 23611, the practical work concerning the respective animal group is described in detail. However, (nearly) nothing is said about how to plan the use of such methods or how to evaluate the results. Despite the fact that sampling for any field study can be different depending on the individual purpose, guidance is needed for monitoring studies in a legal context. Such studies can include the following:

— site-specific risk assessment of contaminated land;

— study of potential side effects of anthropogenic impacts (e.g. the application of chemicals or the building of roads);

— the biological classification and assessment of soils in order to determine the biological quality of soils;

— long-term biogeographical monitoring in the context of nature protection or restoration, including global change [e.g. as in the long-term ecological research project (LTER)].

Spatial studies focusing on environmental and ecological questions require a carefully designed strategy for collecting data (References [31], [65]). Before identifying the optimal design, two issues have to be clarified: what is the objective of the study and what is already known about the survey area? Afterwards, one may select one of the well-known design patterns (e.g. grid sampling, random sampling, clustered sampling or random transects) or prepare a study-specific design. In any case, the field sampling design has to be practical, e.g. the volume of soil to be sampled, depending on the size and distribution of the organisms, has to be manageable (i.e. the smaller the individual animal, the smaller the size), and cost effective.

In studies focusing on soil invertebrates, it is not possible to observe the entire population. Therefore, sampling is done only at a limited number of locations. The main reason for using statistical sound sampling schemes is that such sampling guarantees scientific objectivity and avoids forms of bias such as those caused by judgement sampling. This is especially valuable if the objective is to obtain data that are representative for the whole area. At the same time, statistics-based sampling schemes ensure standardized sampling methods over time, i.e., if the same area is to be re-sampled in the future, the results will be comparable.

The rational for this guidance s on the design of field sampling methods for soil invertebrates takes into consideration the guidance provided in ISO 10381-1 describing soil sampling in general.

The design of microbiological studies is already covered by ISO 10381-6, ISO 14240-1 and ISO 14240-2.

Soil quality — Sampling of soil invertebrates —

Part 6: Guidance for the design of sampling programmes with soil invertebrates

1 Scope

This part of ISO 23611 provides guidance for the design of field studies with soil invertebrates (e.g. for the monitoring of the quality of a soil as a habitat for organisms). Detailed information on the sampling of the most important soil organisms is provided in the other parts of this International Standard (ISO 23611-1 to ISO 23611-5).

This part of ISO 23611 is used for all terrestrial biotopes in which soil invertebrates occur. Basic information on the design of field studies in general is already laid down in ISO 10381-1. This information can vary according to the national requirements or the climatic/regional conditions of the site to be sampled.

NOTE While this part of ISO 23611 aims to be applicable globally for all terrestrial sites that are inhabited by soil invertebrates, the existing information refers mostly to temperate regions. However, the (few) studies from other (tropical and boreal) regions, as well as theoretical considerations, allow the conclusion that the principles laid down in this part of ISO 23611 are generally valid, References [4], [6], [40], [21].

This part of ISO 23611 gives information on site-specific risk assessment of contaminated land, study of potential side effects of anthropogenic impacts (e.g. the application of chemicals or the building of roads), the biological classification and assessment of soils in order to determine the biological quality of soils, and long-term biogeographical monitoring in the context of nature protection or restoration, including global change (e.g. as in long-term ecological research projects).

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10381-1:2002, *Soil quality — Sampling — Part 1: Guidance on the design of sampling programmes*

ISO 10381-2, *Soil quality — Sampling — Part 2: Guidance on sampling techniques*

ISO 10381-3, *Soil quality — Sampling — Part 3: Guidance on safety*

ISO 10381-4, *Soil quality — Sampling — Part 4: Guidance on the procedure for investigation of natural, near-natural and cultivated sites*

ISO 10381-5, *Soil quality — Sampling — Part 5: Guidance on the procedure for the investigation of urban and industrial sites with regard to soil contamination*

ISO 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11074, *Soil quality — Vocabulary*

ISO 11260, *Soil quality — Determination of effective cation exchange capacity and base saturation level using barium chloride solution*

ISO 11272, *Soil quality — Determination of dry bulk density*

ISO 11274, *Soil quality — Determination of the water-retention characteristic — Laboratory methods*

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

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

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 11466, *Soil quality — Extraction of trace elements soluble in aqua regia*

ISO 13878, *Soil quality — Determination of total nitrogen content by dry combustion ("elemental analysis")*

ISO 14869-1, *Soil quality — Dissolution for the determination of total element content — Part 1: Dissolution with hydrofluoric and perchloric acids*

ISO 15709, *Soil quality — Soil water and the unsaturated zone — Definitions, symbols and theory*

ISO 15799, *Soil quality — Guidance on the ecotoxicological characterization of soils and soil materials*

ISO 17616, *Soil quality — Guidance on the choice and evaluation of bioassays for ecotoxicological characterization of soils and soil materials*

ISO 23611-1:2006, *Soil quality — Sampling of soil invertebrates — Part 1: Hand-sorting and formalin extraction of earthworms*

ISO 23611-2, *Soil quality — Sampling of soil invertebrates — Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina)*

ISO 23611-3, *Soil quality — Sampling of soil invertebrates — Part 3: Sampling and soil extraction of enchytraeids*

ISO 23611-4, *Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of free-living stages of nematodes*

ISO 23611-5, *Soil quality — Sampling of soil invertebrates — Part 5: Sampling and extraction of soil macro-invertebrates*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11074 and the following apply.

3.1 Soil biology

3.1.1
biodiversity
variability among living organisms on the earth, including the variability within and between species, and within and between ecosystems

NOTE Also often used as the number and variety of organisms found within a specified geographic region.

3.1.2
community
association of organisms, belonging to different species, families, etc. living at the same time at the same place, i.e. the living portion of an ecosystem

See Reference [42].

3.1.3
invertebrate
term embracing all organisms except the chordates and microflora

NOTE This is not a taxonomic term.

3.1.4
microfauna, mesofauna and macrofauna
way of classifying the soil fauna according to the size (length, diameter) of the individual animals

See Reference [66].

EXAMPLE Important examples of the microfauna are protozoans and nematodes, for the mesofauna collembolans, mites and enchytraeids, and for the macrofauna earthworms and snails.

3.1.5
taxocoenosis
total number of species belonging to the same higher taxonomic unit (e.g. family, order) within a community

3.2 Soil protection

3.2.1
soil quality
capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation

See References [16], [30].

NOTE In more recent definitions, the natural functions of soil are specifically listed: soil as a habitat for organisms, as part of natural systems (in particular nutrient cycles) and for decomposition, retention and filtration, Reference [6].

3.2.2
habitat
sum of the environment of a particular species or community (e.g. in terms of soil properties, land use, climate)

3.2.3
habitat function
ability of soils/soil materials to serve as a habitat for microorganisms, plants, and soil-living animals, and support their interactions (community or biocenosis)

3.2.4
contamination
substance(s) or agent(s) present in the soil as a result of human activity

NOTE There is no assumption in this definition that harm results from the presence of the contaminant.

3.2.5
pollutant
substances which, due to their properties, amount or concentration, cause impacts on soil functions or soil use

3.2.6
reference soil
uncontaminated soil with comparable pedological properties to the soil being studied except that it is free of contamination

3.3 Methods

3.3.1
Geographical Information Systems
GIS
in the strictest sense, a computer system capable of assembling, storing, manipulating, and displaying geographically referenced information, i.e. data identified according to their locations

NOTE Practitioners also regard the total GIS as including operating personnel and the data that go into the system (US. Geological Survey, 2006).

3.3.2
site-specific assessment
evaluation of the quality of a specific-site by using chemical, biological or other methods

3.3.3
environmental risk assessment
process of identifying and quantifying risk (probability that an effect occurs) to non-human organisms and determining the acceptability of these risks

3.3.4
soil function
property of (specific) soils, often used in legal documents

NOTE Usually natural soil functions (e.g. the soil as a habitat for organisms) and anthropogenic soil functions (e.g. soil as a substrate for crop production) are distinguished.

3.3.5
soil organism function
activity provided by individual species or, more often, by interaction of several species or the whole soil community, e.g. nitrogen fixation or organic-matter breakdown.

4 Principle

4.1 General

The design of field studies for the investigation of soil invertebrates differs significantly depending on the respective aim. However, in all cases, it is necessary to take samples since the site and biological populations to be studied are usually too large to be studied in total. In addition, most soil invertebrates live hidden within the soil and/or are too small to be studied directly. The samples collected should be as representative as possible of the site to be characterized but destruction should be kept at a minimum. In addition, the occurrence of material not naturally belonging to the study site (e.g. waste or chemicals) can cause problems when taking samples in multiphase systems such as soils, which contains water, gases, mineral solids and biological material.

The study design (e.g. the position and density of sampling points, time of sampling, and the sampling method) depends mainly on the objectives of the study and on the amount and quality of information already available from the study site (e.g. historical data, personal experience). The design also depends on whether information is needed as an average value (sampling for the spatial mean, e.g. the average number of nematodes) or as a spatial distribution (e.g. sampling for a map showing nematode abundances in relation to soil properties). In addition, the sheer size and the heterogeneity of soil properties, as well as those of the organisms to be sampled shall be taken into consideration. In any case, a list of measurement end points should be compiled for the respective organism group(s) and the main limitations of the sampling method(s) shall also be known. The latter refers mainly to the high natural variability of invertebrate data. The normal statistical tests used by those who take composite samples (microflora, soil properties) or many samples (soil properties) which can be processed more or less automatically, cannot be applied here.

Some consideration should also be given to the degree of detail and precision that is required and also the manner in which the results are to be expressed (e.g. maximum and minimum values in a table, graphical presentations or maps). Appropriate statistical methods for the evaluation of area-related data (including the use of GIS methods) shall be identified as well. It can often be necessary to carry out an exploratory sampling programme before the final study design can be defined in detail. The main points on which decisions shall be made are listed in 4.2, reflecting the logical order of how to proceed a study.

NOTE This clause was written in close consideration with ISO 10381-1.

4.2 Question to be answered when planning a field study

The objective of a study can be established by the following questions:

— Why is such a study going to be performed?

— What information is necessary to answer the questions asked and how can this information be clearly presented?

— Which approach is used for the interpretation of the results?

— How can the study outcome be tailored to the needs of the study sponsor (or stakeholder)?

The preliminary information can be defined by the following questions:

— What is already known about present and historical (especially land-use, management) site and soil characteristics?

— What information is missing? Can it be made available?

— Who is to be contacted for certain (e.g. historical) sources?

— Are there any legal problems such as entering the sites?

— Shall other than biological parameters be measured at the same site and time, i.e. are (negative) interactions of the various sampling programmes to be expected?

— Has the site been visited already?

The strategy of a study can be developed by the following questions:

— How are the delineations in time and space of the area(s) to be investigated determined?

— Which organism groups and measurement end points are appropriate to reach the study objective?

— Which sampling patterns, sampling points, sampling times, depths of sampling should be used?

— Can methods specified in International Standards be employed for all activities?

The decision on sampling and analysis can be made by answering the following questions:

— Can the sampling be done according to the respective International Standard or is there any deviation?

— How is the communication with the personnel responsible for sample presentation and analysis coordinated?

— Which statistical evaluation methods are being employed?

— Does sampling correspond to later data analyses?

— Is it possible to address the right taxonomic level when studying the biological material?

— How is the documentation organized?

The following questions on safety should be answered:

— Are all necessary safety precautions at that site considered?

— Is information concerning landowners, local authorities etc. secured?

— Are the requirements of ISO 10381-3, covering guidance on safety in sampling programmes, as well as those safety issues listed in other parts of this International Standard (ISO 23611-1 to ISO 23611-5 fulfilled?

The following questions on the sampling report should be answered:

— Is there any deviation from the basic content of a study report as specified in this part of ISO 23611?

— Is additional information required?

— How is it ensured that any later deviation from this part of ISO 23611 or the study plan is documented and distributed?

Answers to these questions are given in Clauses 5 to 8.

5 Objectives of sampling

5.1 General

Biological soil investigations address a number of different questions related to the status of invertebrates living in or on the soil (including many different species belonging to different trophic, taxonomic, physiological or functional groups and size classes), often after or under some kind of anthropogenic impact. In the case of ecotoxicological questions, usually laboratory tests are used to study the effects of the impact (e.g. chemicals added to the soil) on invertebrates and thus on the soil quality in general. Such methods are presented in ISO 15799, while the assessment of the test results is given in ISO 17616. Further guidance on sampling, collection, handling and preparation of contaminated soil for biological (i.e. ecotoxicological) testing has currently been prepared by Reference [21]. This is particularly important for the identification and characterization of field reference soils which are necessary for the determination of biological reference values. Examples are provided in Annex A (case studies).

5.2 General remarks

As stated in the Introduction, the principal objectives of sampling soil invertebrates can be distinguished as follows:

— the performance of the site-specific characterization and assessment of contaminated land;

— the study of potential side effects of anthropogenic impacts (e.g. the application of chemicals or the building of roads);

— the biological classification and assessment of soils in order to determine the biological quality of soils;

— long-term biogeographical monitoring in the context of nature protection or restoration, including global change (e.g. as in the long-term ecological research project (LTER)).

To a different degree, all four objectives include the determination of biological reference (or base-line) values, meaning that it shall be clarified which community of soil organisms occurs in a specific soil assuming that there is no anthropogenic impact. Since this precondition is, in many if not all soils, not fulfilled any more, such a “normal” state shall be defined, e.g. by sampling of reference soils. These soils have been selected based on criteria like being representative for certain regions or land-use forms or lack of contamination, Reference [14].

The use of the soil and site are of varying importance depending on the primary objective of an investigation. The results obtained from sampling can indicate a need for further investigation, e.g. detected contamination can indicate a need for identification and assessment of potential hazards and risks. However, assessment of such hazards or risks is not covered by this part of ISO 23611. In addition, capture-recapture methods – while often used in ecology for terrestrial above-ground invertebrates (e.g. spiders, Reference [26]) are rarely used in general monitoring schemes and thus will not be covered in this part of ISO 23611.

Often soil invertebrates are a part of an entire monitoring effort that includes other biological (mainly microbial), as well as pedological, climatic and possibly also agricultural parameters. If such monitoring programmes are performed at regular intervals, permanent sampling sites shall be set up. In such a case, additional efforts are mandatory in order to secure an effective exchange of information. Sampling is usually carried out within the main rooting zone (rarely at greater depths since most soil invertebrates live within the uppermost 30 cm of the soil). Soil horizons or layers may or may not be separately sampled (samples shall be labelled accordingly).

To adequately support legal or regulatory action, particular attention should be paid to all aspects of quality assurance. The guidance given in ISO 10381-5 is particularly relevant. After clarifying the most important pre-conditions, the four groups of main objectives as given above are briefly presented in the following subclauses. However, it should be kept in mind that, in reality, one specific study can fit into more than one of these groups.

5.3 Pre-conditions

Before designing a field study with soil invertebrates, it is highly recommended to characterize the respective area pedologically, Reference [43]. Depending on the principal objectives, it is usually necessary to determine for the body of soil or part thereof

— the nature, concentrations and distribution of naturally occurring substances,

— the nature, concentrations and distribution of contaminants,

— the physical and chemical properties and variations,

— the anthropogenic impact at that site, in particular the land use (including vegetation cover).

It is often necessary to take into account changes in the above-mentioned variables with time and space (vertically, horizontally), caused by either natural (e.g. climatic) or anthropogenic activities.

In addition, pH, particle size distribution, C/N ratio, organic matter and organic carbon content, total nitrogen, cation exchange capacity and water holding capacity of the soil should be measured in accordance with ISO 10390, ISO 10694, ISO 11260, ISO 11272, ISO 11274, ISO 11277, ISO 13878, ISO 11461, ISO 11465, ISO 15709, ISO 17616.

5.4 The performance of the site-specific assessment of contaminated land

When land is contaminated with chemicals and other substances that are potentially acting as pollutants to the environment, it can be necessary to carry out an investigation as a part of a hazard and/or risk assessment. This includes to determine the nature and extent of contamination, to identify hazards associated with the contamination, to identify potential targets and routes of exposure, and to evaluate the environmental risks relating to the current and future use of the site and neighbouring land. A sampling programme for risk assessment can also comply with legal or regulatory requirements and careful attention to sample integrity is recommended. An extensive overview of the benefits and limitations of biological parameters as a component of contaminated land assessment is given in Reference [21].

5.5 The study of potential side effects of anthropogenic impacts

Sampling can be required following an anthropogenic effect such as the input of undesirable material (mainly chemicals) which can be from a point source or from a diffuse source. Another example can be the building of roads. The study design needs again to be developed on a site-specific basis. Sampling can also be required to establish base-line conditions prior to an activity, which might affect the composition or quality of soil.

NOTE Such base-line sampling can also be performed as part of a biological soil classification and assessment (see 5.4).

5.6 The biological classification and assessment of soils in order to determine the biological quality of soils

This is typically carried out at (irregular) time intervals to determine the biological quality of a soil for a particular purpose (e.g. as part of a large-scale screening programme or in the context of a local planning activity). While it has rarely been done so far in terrestrial habitats (except with plants), the information gained here can be used for the preparation of biological soil maps, Reference [8].

NOTE The study of the biological soil quality can also be used for the determination of “base-line conditions” in the context of the assessment of anthropogenic impacts (see 5.3) or of long-term changes such as global warming (5.7).