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Soil quality — Sampling —

Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory

Qualité du sol — Échantillonnage —

Partie 206: Collecte, manipulation et conservation de sols destinés à l'évaluation de paramètres biologiques fonctionnels et structurels en laboratoire

Reference number
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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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

This first edition of ISO 18400-206 cancels and replaces ISO 10381-6:2009, which has been technically revised.

A list of all the parts in the ISO 18400 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

Soils are both complex and heterogeneous because they consist of both living and non-living components occurring in different combinations. Therefore, the condition of the soil, from collection to completion of an experiment, is considered in this document in relation to effects on the soil organism community (i.e. microorganisms, plants and invertebrates). Temperature, water content, availability of oxygen and duration of storage are all known to affect these organisms, and thus the processes they mediate.

Soils can however be used effectively in the laboratory to investigate effects on soil organisms. In this context a distinction is made between microbial communities on the one side and plants and invertebrates on the other side, since the former are sampled as part of a soil sample, while the latter are added to a soil sample (usually only a few selected species which have been identified as test species beforehand). Therefore, this document covers two different issues:

a) It provides guidance on the collection, handling and storage of soil for laboratory use where aerobic microbial activity is the main component of the study. It describes how to minimize the effects of differences in temperature, water content and availability of oxygen on aerobic processes to facilitate reproducible laboratory determinations[1][2].

b) It also provides guidance on the collection, handling and storage of soil for laboratory use where the survival, reproduction, behaviour or growth of invertebrates or plants is the main components of the study. It describes how to minimize the effects of differences in temperature, and the water content as well as the fractionation of soil particles to facilitate reproducible laboratory determinations[1][2].

This document is one of a group of standards dealing with various aspects of site investigation and sampling. It needs to be used in conjunction with the other parts of ISO 18400. The role/position of the standards within the total investigation programme is shown in Figure 1.

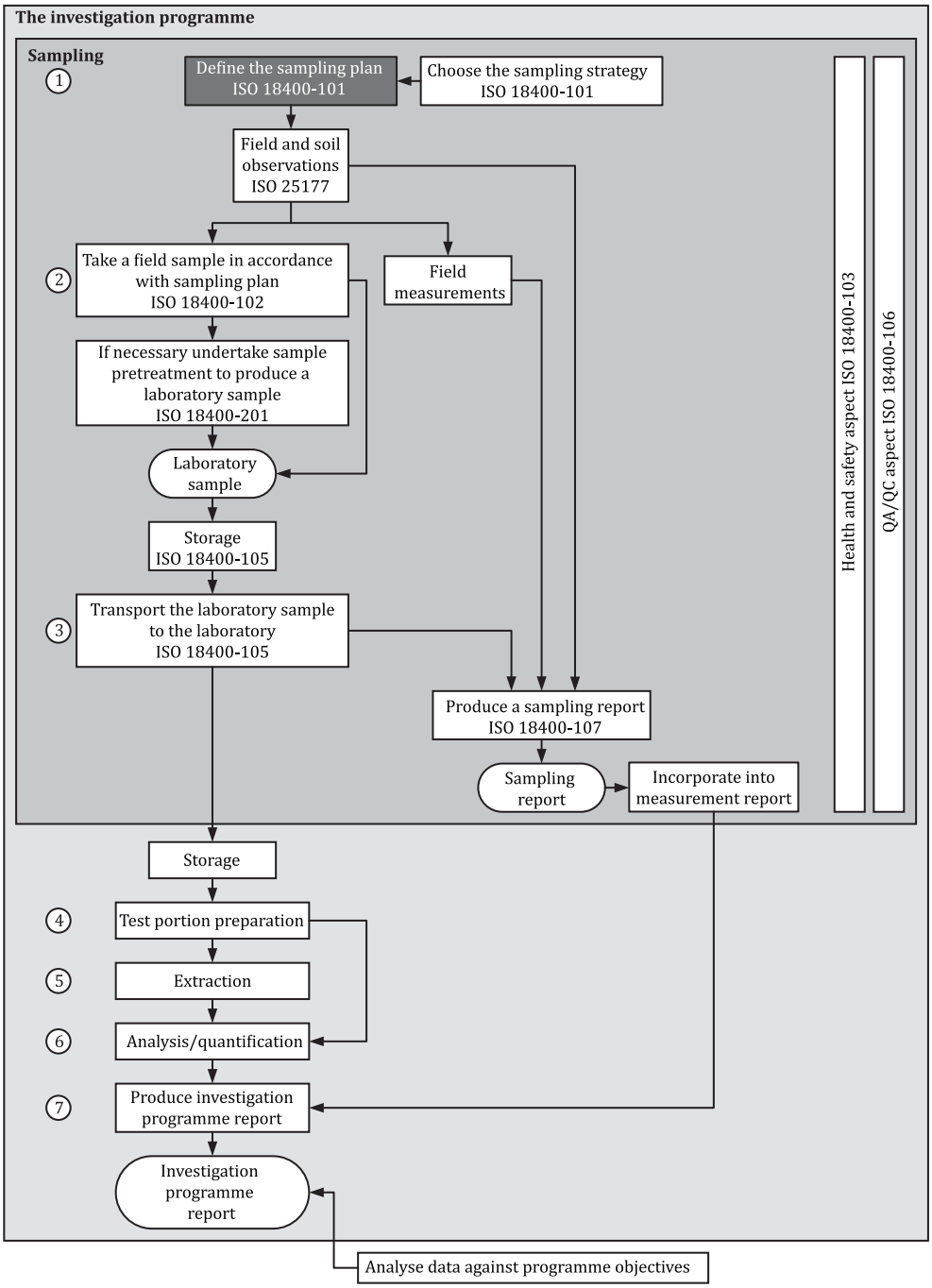

Figure 1 — Links between the essential elements of an investigation programme

NOTE 1 The numbers in circles in Figure 1 define the single steps of the investigation programme.

NOTE 2 Figure 1 displays a generic process which can be amended when necessary.

INTERNATIONAL STANDARD **ISO 18400-206:2018(E)**

Soil quality — Sampling —

Part 206: Collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory

1 Scope

This document provides standard procedures for the collection, handling and storage of soil for subsequent biological testing under aerobic conditions in the laboratory. It applies to the collection, handling and storage for assessing the effects of soil on microorganisms, invertebrates (e.g. survival, reproduction, growth, behaviour) and plants (e.g. development, growth). This document is not applicable to the handling of soil where anaerobic conditions need to be maintained throughout.

This document describes how to minimize the effects of differences in temperature, water content, and availability of oxygen on aerobic processes as well as the fractionation of soil particles to facilitate reproducible laboratory determinations[1][2].

This document is mainly applicable to temperate soils. Soils collected from extreme climates (e.g. permafrost, tropical soils) can require special handling.

NOTE This document does not provide standard procedures on the collection, handling and storage of soil organisms when assessing the structure and function of soil organism communities in the field. Such standard procedures are provided in ISO 23611-1 to ISO 23611-6.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 18400-101, *Soil quality — Sampling — Part 101: Framework for the preparation and application of a sampling plan*

ISO 18400-107, *Soil quality — Sampling — Part 107: Recording and reporting*

ISO 18400-202, *Soil quality — Sampling — Part 202: Preliminary investigations*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
aerobic
descriptive of a condition in which molecular oxygen is freely available

3.2
anaerobic
descriptive of a condition in which molecular oxygen is not available

3.3
water content on a dry mass basis
mass of water evaporating from the soil when dried to constant mass at 105 °C divided by the dry mass of the soil and multiplied by 100

[SOURCE: ISO 11465:1993, 3.2]

4 Procedure for the handling of soil samples to be used in laboratory tests with microorganisms, plants and invertebrates

4.1 Selection of sampling locations

The locations of the sites from which samples are taken should be selected according to the purpose of the study, preliminary information, and on-site conditions. Sampling locations should be representative of the total area to be sampled. These locations should be preferably geo-referenced. Sampling patterns can be based on statistical models, numerical random distributions or systematic patterns as described in ISO 18400-104. For details on the selection of sampling locations for sampling programmes with soil invertebrates see also Reference [3].

NOTE It can be helpful to estimate the uncertainty of the measurement when soil sampling. Appropriate statistical methods depend on the purpose and the design of the sampling (see ISO 18400-104).

Examples of on-site conditions that shall be considered when designing a sampling strategy include local topography, climatic conditions, vegetation cover (especially trees), soil type and/or soil physicochemical characteristics and, if appropriate, the location of a contaminant source (point or non-point) or the direction of contamination. Soil properties as well as soil contamination are often characterized by high variability in time and space. Thus, different designs and statistical methods shall apply depending on the respective study objective.

Depending on the objective of the investigation a sampling pattern is chosen when designing the study and is then applied in the field. Afterwards, preparation of the site includes the establishment of safety measures (see also ISO 18400-103). When sampling if practicable, the locations should be marked so that they can be used for comparative tests or to obtain further samples (e.g. at a later date). This work becomes very time consuming if it is not possible to take a sample at the planned location due to a variety of reasons (e.g. trees, rocks, or access difficulties). Contingency plans for dealing with such situations should be made in advance (ad hoc decisions in the field can lead to a bias). The action taken depends on the circumstances: the point can be ignored, or a nearby substitute location (e.g. within 10 % of grid spacing away from the original location) can be chosen. In every case when a sampling point is re-located, this should be done in accordance with ISO 18400-101 and the reason for relocation shall be clearly indicated in the report.

4.2 Performance of a preliminary survey

A preliminary investigation in accordance with ISO 18400-202 should be carried out prior to any sampling programme, although the effort devoted to it depends on the objective of the investigation. It should always comprise a desk-top study and a site visit. In addition, in the specific context of this International Standard, a limited amount of sampling can be carried out provided that it is safe to do so. The principal objectives of the preliminary study are to gain knowledge about the present condition of the site, and of past activities on the site and adjacent land which can have affected it in order to enable the sampling programme to be designed to be both technically effective and cost effective. In addition, measures shall be identified that protect the health and safety of the investigating personnel and of the environment (see ISO 18400-103).

4.3 Description of field site

Selection of a soil sampling site depends on the purpose of a particular study, and knowledge of the field site history is always desirable. The site should be accurately described (including type and properties of soil, designation of horizons) and its history given. Details of vegetation cover, the morphology of the sampling area (e.g. flat area, slopes, steepness), and of chemical and biological additions or accidental contamination, should be recorded and reported (see ISO 18400-107).

4.4 Sampling conditions

Soil required for studies conducted under laboratory conditions should, if practicable, be sampled in the field with a soil water content which facilitates sieving. Sampling should, unless the purpose of the study requires otherwise, be avoided during or immediately following long periods (e.g. one month) of drought, freezing or flooding. If laboratory tests are to be used for field monitoring, conditions existing in the field should be accepted.

4.5 Sampling methods

The sampling technique depends on the purpose of the study. Appropriate sampling techniques should be selected and applied following the guidance in ISO 18400-102 and ISO 18400-205. If aerobic agricultural soil is required, sampling is usually conducted to the actual ploughing depth. Any surface vegetation cover, moss-covered litter layer, visible roots, large pieces of plant or woody plant litter and visible soil fauna should be removed to minimize the addition of fresh organic carbon to the soil. Organic constituents introduced from roots or other sources can cause unpredictable changes in the activities and composition of the soil microflora and, thus, indirectly also test conditions for plants and invertebrates. If natural soils show distinct horizons, samples should be taken from these horizons. It should be noted that representative samples should be taken from the upper to the lower boundary of the horizon.

4.6 Sample marking

Sample containers should be clearly and unambiguously marked and identified so that each sample can be related to the location from which it was taken. Use of containers which either absorb water from the soil or release materials, e.g. solvents or plasticizers into the soil should be avoided (see ISO 18400-105).

4.7 Transportation conditions

General guidance about transportation and storage of soil samples is given in ISO 18400-105. Samples should be transported in a manner which minimizes changes in the soil water content, and should be kept in the dark with free access of air; a loosely-tied polyethylene bag is generally adequate for this purpose. Extreme environmental conditions should be avoided. The soil should be kept as cool as possible but it is essential that it is not allowed to dry out, become water-logged or become frozen. Exposure to light for extended periods should be avoided as this encourages the growth of algae on the surface of the soil. Physical compaction should be avoided as far as is practicable.

Samples for DNA or RNA analysis shall be frozen quickly in the field using dry ice. During transportation to the laboratory, dry ice shall be used to maintain the temperature of those samples for RNA analysis. Samples for DNA analysis may be transported in a cooling box unless the circumstances are such that dry ice is needed for these as well.

4.8 Soil processing in the laboratory

The soil should be processed as soon as possible after sampling. Vegetation, larger soil fauna and stones should be removed prior to passing the soil through a 2 mm sieve when testing with microorganisms is foreseen. Sieving soil through a 2 mm sieve facilitates gaseous exchange between particles and is therefore recommended for maintaining the aerobic nature of the soil. It also removes small stones, fauna and plant debris. Some organic materials such as humus (litter) layers or peat do not pass easily through a 2 mm sieve and should be sieved in the moist condition through a 5 mm sieve. This necessitates

manual operation and the quality of the material passing the sieve depends on the operator. If the soil is too wet to sieve, it should be spread out, in a gentle air stream where possible, to facilitate uniform drying. The soil should be finger crumbled and turned over frequently to avoid excessive surface drying. Usually, this should be performed at ambient temperature. If drying is required, the soil should not be dried more than necessary to facilitate sieving. Generally, drying of soils is not recommended although air-drying and rewetting is a common physiological stress for the microbial communities in surface soils. It should be kept in mind that plants and invertebrates are more susceptible to drought, meaning that for such tests soil samples could be re-wetted. It has been shown that drying-rewetting events can induce significant changes in microbial C and N dynamics which can last for more than a month after the last stress[4]. Rewetting after drying causes bursts of respiration and growth of distinct populations of bacterial[5]. If further storage is necessary following processing, consideration should be given to the parameters discussed in 4.9.

In case the soil sample is to be used in tests with plants, sieving with a 4 mm sieve is sufficient.

In case of testing soil invertebrates it is often necessary to defaunate the soil beforehand (see Reference [6]).

4.9 Storage conditions and storage periods

It is preferable to use soils as soon as possible after sampling. Any delays due to transportation should be minimized. If storage is unavoidable, appropriate storage conditions for various test objectives are given in Table 1.

When soil samples are stored at (4 ± 2) °C this should be done in the dark with free access of air. A loosely tied plastic bag or equivalent is generally adequate for this purpose. Care should be taken to ensure that the soil is not stored in a quantity which allows anaerobic conditions to occur in the bottom of storage containers. The soil should be processed (see 4.8) before storage in order to ensure stable aerobic conditions. It is essential that the soil is not allowed to dry out or become water-logged during storage. Samples should not be stored on top of one another. If soil samples are stored for longer periods (>three month), freezing of samples at –20 °C, –80 °C or –180 °C can be appropriate although not generally recommended.

For structural analyses of the microflora, storage at 4 °C is not suitable. Soil samples subject to Phospholipid Fatty Acid (PLFA) and DNA analyses should be frozen at –20 °C if not processed immediately. For RNA analyses, samples should be frozen at –80 °C. Shock freezing with liquid nitrogen is recommended for freezing samples subjected to DNA, RNA and PLFA/PLEL analyses.

In case soil samples are to be used in plant and invertebrate tests and do not contain volatile or degradable contaminants, indefinite storage at room temperature is possible as long as the soil samples have been air-dried beforehand (ISO 18512). However, significant changes in nitrogen mineralization and soil pH can happen in some soils, see Reference [13].

Table 1 — Storage conditions and duration for the assessment of biological endpoints when analysis cannot be performed immediately

Test objective	International Standard	**Wet** **4 °C** (or other temperature given here) Days/months	**Wet** **–20 °C**[a] Month/years	**Wet** **–80 °C or liquid nitrogen (–180 °C)**[a] Month/years
Effects on invertebrates	ISO 11267 ISO 11268-1 ISO 11268-2 ISO 15952 ISO 16387 ISO 17512-1 ISO 17512-2 ISO 20963	Three months	—	—
Effects on higher plants	ISO 11269-1 ISO 11269-2 ISO 17126 ISO 18763 ISO 22030 ISO 29200	Three months	—	—
Phospholipid Fatty Acid, Phospholipid ether lipids	ISO/TS 29843-1 ISO/TS 29843-2	7 d	2	10
DNA	ISO 11063	—	2	10
RNA[13]		—	—	10
Biomass — Substrate-induce respiration method — Fumigation-extraction method	 ISO 14240-1 ISO 14240-2	7 d	1	—
Potential ammonium oxidation	ISO 15685	7 d	1	—
Nitrogen mineralization	ISO 14238	7 d	1	—
Microbial soil respiration	ISO 16072	7 d	1	—
Soil respiration curves	ISO 17155	7 d	—	—
Dehydrogenase activities	ISO 23753-1 ISO 23753-2 ISO 18187	7 d 7 d 4 °C ± 2 C 14 d	1	—

Table 1 *(continued)*

Test objective	International Standard	Wet **4 °C** (or other temperature given here) Days/months	Wet **–20 °C**[a] Month/years	Wet **–80 °C or liquid nitrogen (–180 °C)**[a] Month/years
Enzyme activity patterns[27][32]	ISO/TS 22939 ISO 20130	— 15 °C/4 d	—	≥4 month
Denitrifying enzyme activities	ISO/TS 20131-1 ISO/TS 20131-2	7 d	1	—

[a] The soils shall be divided into sub-samples for further investigation before storage. Alternatively, PLFA should be extracted from field-moist soils (<2 mm) immediately after sampling. The extracts can be stored at –20 °C for several months prior to further separation steps and analysis with GC-MS. Shock freezing in liquid nitrogen is recommended before storage at –20 °C or –80 °C.

Symbols:

— No storage possible.

4.10 Pre-incubation

Before the processed soil is used for a specific microbial laboratory test, it should be pre-incubated to allow germination and removal of seeds, and to re-establish an equilibrium of microbial metabolism following the change from sampling or storage conditions to incubation conditions. Pre-incubation conditions depend on the purpose of the study but should approach test conditions as far as is practicable. The period of pre-incubation depends on the purpose of the study, the soil composition and the storage/pre-incubation conditions. A period between 2 d and 28 d is generally adequate.

If samples were frozen, special attention shall be given to the thawing of samples. For the analyses of microbial activity (e.g. soil respiration), a thawing period of one week at 4 °C and another three days at 20 °C are recommended. A thawing period of one day at 20 °C can also be suitable. For DNA, RNA and PLFA/PLEL analyses, the thawing period shall be as short as possible to avoid degradation processes.

Freezing the samples can change the water-holding capacity. Therefore, for such samples the water-holding capacity should be determined after thawing.

5 Sampling report

A sampling report shall be prepared in accordance with ISO 18400-107. It shall, as appropriate:

— facilitate comparison of soil characteristics in soil inventories, land evaluation, etc.;

— include information on the site (location and utilization of the area, soil conditions, conditions of cultivation and climate, etc.);

— list the coordinates of the sample location(s);

— be supplemented by location map(s), field maps, photographs, etc;

— provide details of the condition of the sampled area at the time of sampling (e.g. crop type);

— include a detailed description of the study objective used.

The sampling report prepared by the field staff should contain the following details:

— sample designation and number (identical to the marking on the sample container);

— date of sampling;

— information on the site (e.g. location, land use, soil type (according to WRB classification) textural class, weather conditions);

— description of soil profile in special cases;

— information on the procedures used (field pattern, sampling equipment, depth of sampling, number of increments composite samples etc.);

— information on storage and transport;

— information on the time and place of delivery to the laboratory;

— identification of sampler;

— confirmation of receipt by laboratory.

In addition, chain-of-custody forms can be important when samples are required for legal purposes.

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