INTERNATIONAL STANDARD

ISO 10381-6

Second edition
2009-03-15

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

Qualité du sol — Échantillonnage —

Partie 6: Lignes directrices pour la collecte, la manipulation et la conservation, dans des conditions aérobies, de sols destinés à l'évaluation en laboratoire des processus, de la biomasse et de la diversité microbiens

Reference number
ISO 10381-6:2009(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

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 10381-6 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

This second edition cancels and replaces the first edition (ISO 10381-6:1993), Subclauses 3.6, 3.7, 3.8 and Clause 4 of which have been technically revised. Table 1 has been added.

ISO 10381 consists of the following parts, under the general title *Soil quality — Sampling*:

— *Part 1: Guidance on the design of sampling programmes*

— *Part 2: Guidance on sampling techniques*

— *Part 3: Guidance on safety*

— *Part 4: Guidance on the procedure for investigation of natural, near-natural and cultivated sites*

— *Part 5: Guidance on the procedure for the investigation of urban and industrial sites with regard to soil contamination*

— *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*

— *Part 7: Guidance on sampling of soil gas*

— *Part 8: Guidance on sampling of stockpiles*

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, should be considered in relation to effects on the soil microflora. Temperature, water content, availability of oxygen and duration of storage are all known to affect the soil microflora, and thus the processes they mediate.

Soils can however be used effectively in laboratory systems to investigate microbially-mediated processes, provided that the dynamics of the living microflora are appreciated. This part of ISO 10381 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 [10], [11].

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

1 Scope

This part of ISO 10381 provides guidance on the collection, handling and storage of soil for subsequent testing under aerobic conditions in the laboratory. The recommendations in this document are not applicable to the handling of soil where anaerobic conditions are to be maintained throughout.

This part of ISO 10381 is mainly applicable to temperate soils. Soils collected from extreme climates (e.g. permafrost, tropical soils) may require special handling.

2 Terms and definitions

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

2.1
aerobic
descriptive of a condition in which molecular oxygen is freely available

2.2
anaerobic
descriptive of a condition in which molecular oxygen is not available

2.3
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

[ISO 11465:1993, 3.2]

3 Procedure

3.1 Selection of sampling locations

The locations of the sites from which samples are taken should be selected according to the purpose of the study.

These locations should be identified and recorded, e.g. on a map by reference to easily recognizable static objects or by using a detailed map reference or by GIS. If practicable, the locations should be marked so that they may be used for comparative tests or for obtaining replicate samples.

3.2 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 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.

3.3 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. 1 month) of drought, freezing or flooding. If laboratory tests are to be used for field monitoring, conditions existing in the field should be accepted. Soil samples may be frozen before investigations of, for example, ammonium oxidations.

3.4 Sampling methods

The sampling technique depends on the purpose of the study. 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. If natural soils show distinct horizons, samples should be taken from these horizons.

3.5 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.

3.6 Transportation conditions

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 or become water-logged. 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 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.

3.7 Soil processing

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. 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 moor 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 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 [12]. Rewetting after drying causes bursts of respiration and growth of distinct populations of bacteria [15]. If further storage is necessary following processing, consideration should be given to the parameters discussed in 3.8.

3.8 Storage conditions and storage periods

Samples should be stored in the dark at (4 ± 2) °C 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 3.7) 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. It is preferable to use soils as soon as possible after sampling. Any delays due to transportation should be minimized. If storage is unavoidable, this should not exceed three months unless there is evidence showing continued microbial activity. Soil samples subject to DNA analyses should be frozen at −20 °C if not processed immediately. For RNA analyses, samples should be frozen at −80 °C.

If soil samples need to be stored for longer periods (> three months), freezing of samples at −20 °C, −80 °C or −180 °C may be appropriate although not generally recommended. Examples for appropriate storage conditions for various test objectives are given in Table 1. It has been shown for a number of soils from temperate climates that storage at −20 °C for up to 12 months does not inhibit microbial activity (e.g. ammonium oxidation). Moreover, soil samples for Phospholipid Fatty Acid (PLFA) and DNA analyses can be stored at −20 °C for one to two years. Samples for rRNA analyses can be stored at −80 °C for the same period. Shock freezing with liquid nitrogen is recommended for freezing samples subjected to DNA, RNA and PLFA/PLEL analyses.

Longer storage periods are mainly needed if the influence of added pollutants on soil microbes and microbial processes is tested with the same soil material or if the community structure (PLFA, DNA, RNA) of soils is evaluated at a distinct point of time during the year. In these cases, the time needed for analyses can easily exceed three months (chemical, pollutant testing). For structural analyses of the microflora, storage at 4 °C is not suitable.

Table 1 — Storage conditions for the assessment of aerobic microbial processes when analysis cannot be performed immediately

Test objective	International Standard	Wet 4 °C months	Wet –20 °C[a] years	Wet –80 °C or liquid nitrogen (–180 °C)[a] years
PLFA, PLEL		—	2	10
DNA		—	2	10
RNA		—	—	10
Biomass – Substrate-induced respiration method – Fumigation-extraction method	 ISO 14240-1 ISO 14240-2	3	1	10
Potential ammonium oxidation	ISO 15685	3	1	10
Nitrogen mineralization	ISO 14238	3	1	10
Microbial soil respiration	ISO 16072	3	1	10
Soil respiration curves	ISO 17155	3	1	10
Dehydrogenase activity	ISO 23753-1 ISO 23753-2	3	1	—
symbols: — = no storage possible.				

[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.

3.9 Pre-incubation

Before the processed soil is used for a specific 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 might 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.

4 Sampling report

The detailed sampling report depends on the sampling objectives but, in general, the following data should be reported:

a) a reference to this part of ISO 10381;

b) location of the site (sufficiently precise for another person to find it without further guidance);

c) a comprehensive description of the relevant details and features of the site;

d) history of the site, including previous use and any known accidental or intentional chemical or biological additions;

e) the date and time of sample collection;

f) the weather conditions at the time or immediately prior to sampling, including air temperature, rainfall, sunshine, cloud, etc.;

g) the precise location from which the sample was taken;

h) the type of device used to take the sample;

i) whether or not the sample needed drying before sieving;

j) the number of samples taken, the area of sampling plot or sampling area;

k) the depth of sampling;

l) whether the individual soil samples were kept separate or pooled into composite samples;

m) the time elapsed between sampling, transport and post-sampling handling;

n) any other factor that might influence the results of subsequent testing.