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Soil quality — Guidance on long and short term storage of soil samples

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National foreword

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Foreword

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ISO 18512 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 2, *Sampling*.

Introduction

Many soil investigation programmes require that soil samples be stored for future use. The choice of storage conditions may determine whether or not the samples will be suitable for the intended future use. This International Standard gives guidance on choosing conditions for storage of soil samples.

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Soil quality — Guidance on long and short term storage of soil samples

1 Scope

This International Standard gives guidance on how to store and preserve soil samples for laboratory determinations and how to prepare them for analysis after storage. Special emphasis is given to maximum storage times as a function of different storage conditions.

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.

EN 15192:2006, *Characterisation of waste and soil — Determination of Chromium(VI) in solid material by alkaline digestion and ion chromatography with spectrophotometric detection*

ISO 10301, *Water quality — Determination of highly volatile halogenated hydrocarbons — Gas-chromatographic methods*

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 10382, *Soil quality — Determination of organochlorine pesticides and polychlorinated biphenyls — Gas-chromatographic method with electron capture detection*

ISO 10390, *Soil quality — Determination of pH*

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

ISO 11048, *Soil quality — Determination of water-soluble and acid-soluble sulfate*

ISO 11074, *Soil quality — Vocabulary*

ISO 11259, *Soil quality — Simplified soil description*

ISO 11261, *Soil quality — Determination of total nitrogen — Modified Kjeldahl method*

ISO 11263, *Soil quality — Determination of phosphorus — Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution*

ISO 11265, *Soil quality — Determination of the specific electrical conductivity*

ISO 11266, *Soil quality — Guidance on laboratory testing for biodegradation of organic chemicals in soil under aerobic conditions*

ISO 11267, *Soil quality — Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants*

ISO 11268-1, *Soil quality — Effects of pollutants on earthworms (*Eisenia fetida*) — Part 1: Determination of acute toxicity using artificial soil substrate*

ISO 11268-2, *Soil quality — Effects of pollutants on earthworms (*Eisenia fetida*) — Part 2: Determination of effects on reproduction*

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

ISO 13877, *Soil quality — Determination of polynuclear aromatic hydrocarbons — Method using high-performance liquid chromatography*

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

ISO 14154, *Soil quality — Determination of some selected chlorophenols — Gas-chromatographic method with electron-capture detection*

ISO 14238, *Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes*

ISO 14240-1, *Soil quality — Determination of soil microbial biomass — Part 1: Substrate-induced respiration method*

ISO 14240-2, *Soil quality — Determination of soil microbial biomass — Part 2: Fumigation-extraction method*

ISO 14255, *Soil quality — Determination of nitrate nitrogen, ammonium nitrogen and total soluble nitrogen in air-dry soils using calcium chloride solution as extractant*

ISO/TS 14256-1, *Soil quality — Determination of nitrate, nitrite and ammonium in field-moist soils by extraction with potassium chloride solution — Part 1: Manual method*

ISO 14507, *Soil quality — Pretreatment of samples for determination of organic contaminants*

ISO 15009, *Soil quality — Gas chromatographic determination of the content of volatile aromatic hydrocarbons, naphthalene and volatile halogenated hydrocarbons — Purge-and-trap method with thermal desorption*

ISO 15473, *Soil quality — Guidance on laboratory testing for biodegradation of organic chemicals in soil under anaerobic conditions*

ISO 15685, *Soil quality — Determination of potential nitrification and inhibition of nitrification — Rapid test by ammonium oxidation*

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

ISO 15903, *Soil quality — Format for recording soil and site information*

ISO 15952, *Soil quality — Effects of pollutants on juvenile land snails (Helicidae) — Determination of the effects on growth by soil contamination*

ISO 16072, *Soil quality — Laboratory methods for determination of microbial soil respiration*

ISO 16387, *Soil quality — Effects of pollutants on Enchytraeidae (*Enchytraeus *sp.) — Determination of effects on reproduction and survival*

ISO 16703, *Soil quality — Determination of content of hydrocarbon in the range C10 to C40 by gas chromatography*

ISO 17155, *Soil quality — Determination of abundance and activity of soil microflora using respiration curves*

ISO 20963, *Soil quality — Effects of pollutants on insect larvae (*Oxythyrea funesta*) — Determination of acute toxicity*

ISO 22030, *Soil quality — Biological methods — Chronic toxicity in higher plants*

ISO 22155, *Soil quality — Gas chromatographic quantitative determination of volatile aromatic and halogenated hydrocarbons and selected ethers — Static headspace method*

ISO 23753-1, *Soil quality — Determination of dehydrogenase activity in soils — Part 1: Method using triphenyltetrazolium chloride (TTC)*

ISO 23753-2, *Soil quality — Determination of dehydrogenase activity in soils — Part 2: Method using iodotetrazolium chloride (INT)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 11074 and ISO 11259 apply.

In this International Standard, the term “refrigeration” refers to a temperature of 4 °C ± 2 °C. The term “freezing” refers to a temperature lower than –18 °C.

4 General comments on soil storage

Many studies involve the collection of soil samples in the field, followed by laboratory determination of various properties of the collected samples. In general, the samples are taken at the site being investigated, mixed or otherwise treated at the site, packed in containers and then transported to the laboratory. Upon arrival at the laboratory, the samples may again be treated before being sent for analysis. Some samples may be stored directly for later analysis. After analysis, the remaining part of the samples may be discarded or stored. The samples are stored when there is a need for further analysis, either because there is a need for checking parameters already determined or there is a need for making additional determinations in the future.

In practice, there are two main situations in which sample storage is relevant.

— Routine testing of soil samples, e.g. by environmental laboratories, where soil samples typically are stored for a few weeks after sampling in order to carry out some additional tests, or in order to confirm results found earlier.

— Situations in which samples have to be stored for a long period, sometimes over decades, e.g. monitoring programs, reference materials, or research programs in which degradability is tested.

Both these situations fall within the scope of this International Standard.

The conditions for storage should be selected carefully at all stages, from the point of taking the sample onwards. As an unexpected delay in transport may occur, this guidance should be applied even if the planned transportation time is short. Examples of storage conditions to be considered are light, temperature, humidity, accessibility, duration of storage, type of container and amount of sample to be stored. Documentation of the samples and the storage conditions is also important. Risk and security problems should be considered. Well-designed storage conditions are particularly important in large-scale studies, such as monitoring, where the number of samples may become quite large over the years. Incorrectly chosen storage conditions may lead to high costs and may render the samples unfit for future use.

The effect of storage on biodiversity has been considered only with respect to microbiological diversity.

Radioactive change caused by loss or gain of radioactive matter should be considered in connection with the respective compounds. Radioactive decay is generally not affected by storage and is not treated in this International Standard.

5 Change in soil properties during storage

It is helpful to consider the principal biological, chemical and physical phenomena that may cause changes in the samples:

— change in water content;

— biological activity;

— evaporation or precipitation of volatile substances;

— chemical reactions with the atmosphere;

— reactions with the sample container.

Unacceptable changes in soil parameters may occur if these phenomena are not controlled by a proper choice of storage conditions. However, controlling all these phenomena in all samples for a long period of time may turn out to be very costly or impossible. It is therefore important to design the storage conditions to fit the objectives of the study.

It is worth noting that some parameters, for example, the contents of some volatile substances, may not be measurable after storage, regardless of storage conditions. In such cases, serious consideration for the future need for data on such parameters should be given at the outset, and the analysis program adapted accordingly.

6 Storage conditions

6.1 General

This clause contains a list of storage conditions that shall be determined when designing the storage programme.

6.2 Light

Light conditions affect the content of some substances, particularly organics. This should be considered and taken care of, e.g. by using brown glass bottles or keeping the samples in total darkness.

6.3 Temperature

The choice of temperature is always very important as the temperature affects the biological activity in the samples. Temperature is therefore a major factor in the design of a storage facility. In some cases, room temperature will be appropriate but, in many cases, refrigeration or freezing may be required to reduce the biological activity. In very special cases, the temperature of liquid nitrogen will be required.

The need for storage of a few samples at – 80 °C or a lower temperature should be considered, e.g. storage of higher quality reference samples at – 80 °C or at a lower temperature, in order to demonstrate whether or not samples stored at low temperatures are stable.

6.4 Humidity

Moisture will induce microbiological activity or chemical changes in soil samples unless the temperature is very low. The control of humidity is therefore important.

When the samples are not kept in airtight containers, the humidity of the storage facility shall be kept low all year round.

If airtight containers are used, the sample humidity will not change during storage. In this case, it is necessary to ascertain that the original humidity of the samples is low enough to prevent microbiological activity.

6.5 Accessibility, security, documentation and quality control

If the samples are to be analysed urgently, or repeatedly, the storage facility should be easily accessible from the laboratory. This will reduce the time and the risk for quality deterioration during transport to the laboratory.

Security issues, such as fire, theft and destruction, are also important, particularly for samples of great value.

Documentation (see ISO 15903), proper labelling and elimination of cross-contamination are other safety issues to be addressed.

Samples from contaminated land should always be regarded as hazardous and handled accordingly.

A relevant quality control (QC) programme should be introduced. A (certified) reference sample may be used, or a prior analysis on one or more of the freshly taken samples.

6.6 Duration of storage

The required duration of storage is an important element in the storage conditions. As mentioned in Clause 4, some samples are stored only for a few weeks (e.g. for routine environmental testing), other samples for a long period of time. Well-documented long-term “reference” soil samples collected at regular intervals, over many years, could be used to determine the magnitude of any changes in important soil properties. There may also be legal requirements on the duration of storage.

The need for a long duration of storage should always be evaluated versus the cost of storage and documentation.

6.7 Containers and quantity of sample stored

The containers should be carefully chosen regarding the construction material, type of sealing and size. Relevant functions should be validated, e.g. the protection from contamination and the ability to keep the sample protected from light or air. Appropriate cleaning or sterilizing procedures shall be followed.

Many plastic containers will become brittle after five to ten years and glass containers are preferred. However, if the samples contain a high content of water, as many clay samples do, the glass may crack on freezing. The risk of cracking on freezing can be reduced by partial filling of the bottles.

The amount of sample to be stored should be considered. The amount required depends on the planned determinations and may be difficult to calculate. Unless the material is very costly or the need for reanalysis is very unlikely, it is wise to store enough material for at least five determinations of the parameter requiring the largest sample size. In addition, storage of at least 50 g is recommended in order to allow homogeneity.

Once a soil is frozen, it is very difficult to sub-sample for a repeat analysis. Thus, it is wise to freeze a number of smaller sub-samples. Care should be taken to guarantee the homogeneity when sub-samples are prepared.

6.8 Preparing the samples after storage

Appropriate procedures for preparing the samples after storage will depend on the storage conditions and the determinations. It is not possible to give a general specification. Existing standards (e. g. ISO 11464) should be considered.

When a non-frozen soil sample is stored for a long period of time, a vertical redistribution of particles may occur. Remixing in a suitable mixer is advisable. For large samples, remixing in a mixer may not be sufficient. Mixing by spreading the sample in a thin layer on a plastic foil, then repeatedly folding the layer and spreading it out again, is recommended.

For frozen samples, thawing conditions shall be defined, as they can influence the determination of biological, microbiological and organic parameters. The samples shall be thawed in their original bags or containers.

7 Stepwise scheme

This International Standard is based on a simple stepwise procedure that enables an informed decision on the choice of conditions for storage and preservation which can be considered in advance of the collection of samples in the field.

The steps are given below.

Step A Consideration of the need for further analysis and duration of storage (Clause 8).

Step B Consideration of parameters presently relevant to the study (Clause 9).

Step C Consideration of parameters that may be of interest in the future (Clause 10).

Step D Consideration of how each of these parameters may be affected by storage conditions (Clause 11).

Step E Design of storage conditions to prevent changes in sample properties (Clause 12).

Step F Design of documentation and labelling scheme including sample management (Clause 13).

Step G Estimation of costs for storage and documentation and comparison of these costs with available or expected funding (Clause 14).

8 Step A: Consideration of need for further analysis and duration of storage

If there is no need for further analysis, there is little cause for sample storage. There are many reasons for further analysis which should be considered. Examples:

— resources may not be available at the time of sampling for all the determinations required;

— methods for all the determinations required may not be available at the time of sampling;

— there may be uncertainty in the method chosen which calls for possible further analysis;

— legal requirements for possibility of further analysis may exist;

— expansion of the analytical programme depending on the results of preliminary analyses should be considered.

9 Step B: Consideration of parameters currently relevant to the study

The soil properties of interest should be part of the study programme for which the samples have been collected. In order to make an informed decision on the storage conditions, it is important to go through this list of relevant parameters.

Existing ISO soil standard parameters should usually be given high priority.

10 Step C: Consideration of parameters which may be of interest in the future

The possibility of new parameters being included in the study at a later date should always be considered.

11 Step D: Consideration of how each of these parameters may be affected by storage conditions

11.1 General

The consideration of how each of the parameters defined in Steps B and C may be affected by storage conditions is a critical step. If the number of parameters is large, step D may be time consuming. In monitoring programmes, there may be 50 or more parameters.

Grouping of the parameters, as shown in 11.3-11.4, is strongly recommended.

This International Standard does not aim to give specific information on the effect of all storage conditions on all possible parameters. The information presented below is a selection based on common requirements.

Due to the small number of studies, there is sometimes a lack of scientific evidence on how long-time storage affects the parameters to be measured. General experience has been accumulated in Table A.1 in Annex A.

11.2 Soil characteristics

11.2.1 Electrical conductivity

The electrical conductivity reflects the salt content of the soil and should not vary much with time.

11.2.2 pH

In air-dried or frozen soil samples, the pH is not expected to change during storage for several years. Studies have shown that no difference in pH could be observed in samples stored for up to 6 months at temperatures between – 18 °C and – 150 °C, liquid nitrogen [7]

11.2.3 Water-holding capacity

Freezing may change the water-holding capacity of soil. If the water-holding capacity of the sample after storage is of interest, the value has to be remeasured.

11.2.4 Structural stability

Samples taken for assessment of the structural stability of soil cannot be frozen, as freezing disrupts the structure of the soil. If the stability in the field state is required, samples cannot even be dried. On this basis, they should be treated without delay.

11.3 Chemical parameters

11.3.1 Metals and trace elements

Provided that the air-dried soil samples have been stored at room temperature in glass containers or in sealed polyethylene bags further protected by dust-tight plastic containers, the storage time is generally at least 7 years for subsequent extraction by quite strong and strong extracting solutions for the determination of the macronutrients K, Mg, Ca and the trace elements As, Be, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, V, Zn. Examples of quite strong and strong extracting solutions are Mehlich 2 and Mehlich 3 extractants, 2 mol/l HNO_3 and *aqua regia*. Cr(VI) is stable for 30 days after sample collection in wet soil at refrigeration. The risk of loss of mercury in elemental form or in volatile compounds shall be considered.

When using a weak extracting solution (e.g. 0,01 mol/l $CaCl_2$) the soil samples shall be refrigerated to ensure long-term stability. Some measured extractable nutrient concentrations were found to be changed by storage at elevated temperatures [3].

11.3.2 Total nitrogen and nitrogen compounds

Nitrogen in soil appears in inorganic forms, mainly nitrate and ammonium ions, and in organic forms as organic compounds, some of which are a part of the microbial soil biomass. The dynamic balance between these forms cause a major problem when storing soil samples for determination of nitrogen and its compounds. The most significant factor affecting this balance is the soil biological activity that is dependent on the aeration, temperature and humidity of the soil. All these parameters may vary during the sampling, transport, sample treatment, etc., and the determined concentrations of the individual nitrogen forms may not be relevant to the actual concentrations at the sampling site. To reduce the risk for changes in the inorganic forms of nitrogen, the samples should be kept frozen.

A sample taken at a soil temperature below 4 °C can be analysed either immediately after transporting to the laboratory, or after storage in the refrigerator at a *maximum temperature* of 4 °C for no more than 7 days, or after storage below – 18 °C for no more than 6 weeks. For $N\text{-}NH_4$ analysis, especially, it is necessary to decrease the sample temperature to below 4 °C, as quickly as possible. For total nitrogen, no significant changes were observed after long-term storage under air-dried conditions for up to 69 years of soil samples at the Rothamsted experimental station.

The storage time of soil samples in a dry state is different for both inorganic forms of nitrogen. For $N\text{-}NO_3$, no change was observed after at least 25 weeks of storage at room temperature in closed polyethylene bags, and for $N\text{-}NH_4$, no change was observed within 6 weeks of storage [8].

In accordance with ISO/TS 14256-1 for determination of nitrate, nitrite and ammonium in field-moist soils by extraction with potassium chloride solution, samples may be stored under refrigeration provided that the analysis is made within 3 days. The samples should be stored frozen if a longer storage time is needed.

When samples are dried by air-drying or some other drying methods, the moist soil sample will remain at an optimal temperature for microorganism activity for some time. This can cause a significant increase of the amounts of $N\text{-}NO_3$ and $N\text{-}NH_4$. The higher the drying temperature, the higher the determined content of the $N\text{-}NH_4$ form will be. For $N\text{-}NO_3$ no changes are expected if samples are stored below 4 °C or a drying temperature of 105 °C is used. If the contribution of the ammonium form can be neglected, samples can be dried at a higher temperature (105 °C) before analysis, without influencing the amount of $N\text{-}NO_3$.

11.3.3 Total phosphorus and phosphorus compounds

Provided that the air-dried sub-samples have been stored at room temperature in glass containers or in sealed polyethylene bags further protected by dust-tight plastic containers, the storage time of soil samples for applied extractions by quite strong and strong extracting solutions (e.g. Mehlich 2, Mehlich 3 [6], 2 mol/l HNO_3, *aqua regia*) for the determination of phosphorous has been found to be at least 7 years.

11.3.4 Other inorganic parameters (chloride, sulfate, fluoride, cyanide, sulfide)

In general, these inorganic parameters are expected to be quite stable over time. As sulfide will react with air to form sulfate, it is necessary to store samples for sulfide determination in the absence of air.

The analysis for the German environmental-specimen bank indicates no significant difference in concentrations for phosphate, sulfate and chloride between frozen samples (–18 °C, –150 °C, liquid nitrogen) and a control stored at 4 °C for short-term storage up to 6 months [7]

11.3.5 Volatile and semi-volatile organic substances

General information on sampling and pretreatment for the determination of volatile and semi-volatile organic substances is given in ISO 14507 and ISO 15009.

It is very difficult to store soil samples for the determination of volatile and semi-volatile organic substances. Completely airtight containers may be very expensive or may not exist at all. Furthermore, volatiles can evaporate even within an airtight sample container because of the air volume existing between the soil particles if the soil sample is not completely saturated with water or other liquid. It is therefore generally advised to carry out the analysis for these substances as early as possible after sampling and to avoid storage of the samples.

If storage cannot be avoided, it is necessary to use containers made of a material that is not penetrable by the substance. The containers should be designed in such a way that they may be sealed completely and opened with safety. They should be filled so that headspace is minimal. Storage at low temperature should always be considered in order to lower the vapour pressure. Immersion of the samples in a solvent, e.g. methanol (see ISO 22155), will reduce the loss of volatile analytes so that the samples can be stored in sealed bottles in the dark for about 10 days, even at room temperature. Otherwise, losses will occur within a few days. This immersion will also eliminate biological activity.

The immersion of the samples in a solvent may be performed at the moment of sampling in the field by using a small core sampler, see the example in Figure 1. The outer diameter of the sampler shall be smaller than the opening of the wide-neck screw-cap bottle. The sample bottle may be about 100 ml. The screw cap of the bottle shall have an inlay, like a septum, coated by polytetrafluoroethylene (PTFE). The lip, the neck and the screw of the bottle shall be kept thoroughly clean and free of any particles. The core is submerged immediately into the sample bottle containing a mass of solvent that is already known. For a sample size of 25 g to 50 g of soil, 25 ml to 50 ml of methanol is suitable. The core should be protected from the open air prior to submerging.

The behaviour of volatile organic compounds is a strictly limiting factor in handling soil material. No mixing, dividing or sieving should be performed in the field or in the laboratory. It is not possible to take bulk samples from which divided or separated parts are taken later for reanalysis. Samples stored for later investigations shall be collected as a sufficient number of separate individual samples from each sampling point. An alternative is to store sample extracts under controlled conditions. Composite samples should be prepared only by combining several individual cores within one bottle or by mixing aliquots of the organic extracts.

WARNING — It should always be remembered that many volatile substances are toxic.

Figure 1 — Example of a core sampler made of metal with a PTFE syringe plug for sampling soils containing volatile organic compound (Inst. Berghof, Tübingen)

11.3.6 Non-volatile organic substances

Soil samples containing non-volatile organic substances are relatively stable. The boiling point for these compounds is typically over 300 ºC, and most of them are described as persistent in the environment. However, many of these substances are affected by biological activity in the soil. Therefore, storage in the frozen state should always be considered for long-term storage.

When analysing organic non-volatile contaminants, it is recommended to use containers made of glass, PTFE, or stainless steel equipped with cups made of or lined with PTFE. Storage before the analyses should take place in the dark under refrigeration.

11.4 Biological tests

Biological tests can be separated into microbiological and biochemical tests, soil fauna tests, plant tests, biodegradation tests and tests for the ecotoxicological characterisation of soils and soil materials. Storage conditions required for soils to be tested with these methods vary widely, and depend on the organism or parameter to be tested.

11.4.1 Microbiological tests

The selection of storage conditions is very important for microbiological tests as the active soil microflora decreases with increasing storage time, even at low temperatures. The rate of decrease depends on the composition of the soil and the microflora. For more detailed information, refer to the latest version of ISO 10381-6.

As a rule, samples for microbiological analyses under laboratory conditions should be taken in the field at a soil water content that facilitates sieving. In the laboratory, the soil should be processed (sieving) as soon as possible after sampling. Samples should be stored in the dark under refrigeration with free access of air, unless anaerobic phenomena are of interest. It is preferable to use soils as soon as possible after sampling. If storage is unavoidable, this should not exceed 3 months, unless evidence showing continued microbial activity is provided.

If soil samples have to be stored for periods longer than 3 months, freezing of samples or storing them at even lower temperature (–80 °C or –150 °C) may be appropriate, although not generally recommended. 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 analyses of phospholipid fatty acid (PLFA) and DNA can be stored at –20 °C for 1 –2 years. Samples for rRNA analyses can be stored at –80 °C for the same period, provided the samples are frozen immediately at –180 °C (shock freezing with liquid nitrogen).

Longer storage periods are mainly needed if the influence of added pollutants on soil microbes and microbial processes shall be tested with the same soil material, or if the community structure [2] (PLFA, DNA, RNA) of soils shall be evaluated at a distinct point of time during the year. In these cases, the time needed for analyses can exceed easily three months (chemical, pollutant testing). It is recommended that samples be stored at 4 °C during these studies.

After storage under freezing conditions, special attention should be given to the thawing of samples. Freeze-thaw cycles can increase the availability of organic matter to microorganisms [2]. For analyses of microbial activity (e.g. soil respiration), a thawing period of one day under refrigeration followed by incubation for three days under room temperature is recommended.

Drying of soils is not generally recommended for biological tests. It has been shown that drying-rewetting events can induce significant changes in microbial carbon and nitrogen dynamics which can last for more than a month after the last stress [1]. Rewetting after drying causes bursts of respiration and growth of distinct populations of bacteria [5].

11.4.2 Biodegradation tests

When testing the biodegradation of organic chemicals in soils (ISO 11266, ISO 15473), storage of soils should be avoided as far as possible because the activity of soil microorganisms will decrease in the course of time. Storage under refrigeration for up to three months is permissible. For the assessment of the anaerobic degradation of chemicals, access of oxygen should be avoided during storage.

11.4.3 Ecotoxicological testing of soils and soil materials

There are no specific recommendations for soil storage with respect to soil fauna and higher plant tests in ISO 15799. Storage of soil samples for the soil fauna test under the same conditions as for testing of microbes and microbial processes is recommended. The reason for doing this is that the availability and effectivity of pollutants is essentially governed by microbial activity. The same is also true for plant testing. In addition, the nutrient supply of test soils should be considered, especially if unknown contaminated soils are tested to avoid false negative results.

Generally, sieved samples should be stored in darkness. For microbial analyses, soils and soil materials should be handled as given in 11.4.1. For terrestrial analyses (e.g. plant tests, earthworm tests), samples can be stored under refrigeration for about three months.

Soils that are used as a dilution or a reference medium can be stored air-dried at room temperature for an unlimited time period.

For testing the leaching potential/retention function of soils and soil materials, water extracts for aquatic tests should be prepared immediately after sieving. If tests cannot be performed within 10 days (storage of the extracts under refrigeration in the dark) new extracts should be made.

12 Step E: Design of storage conditions required to avoid change in sample properties

The storage conditions considered for the various parameters in the previous step should be brought together into a design of storage conditions. If conflict situations occur, it may be necessary to store two or more sub-samples at different storage conditions.

13 Step F: Design of documentation and labelling scheme including sample management

As the samples may be stored for many years, it is necessary to consider at the outset how the information about the samples should be documented and how the samples should be managed to maintain the chain of custody. The relevant International Standard for recording soils and site information is recommended for the initial information (ISO 15903). Information about the history and handling of each sample should be added. Such information may relate to the movement of the sample from one location to another, or to sub-sampling from the sample for analysis. It is also important to decide how the documentation is to be stored.

Degradation of labels can be disastrous. Putting a label inside the container can result in the label being destroyed by the soils. Labels stuck onto the outside of the container may fall off. Painting, stamping or engraving reference numbers or codes both on the outside of the containers and the lids is advisable.

14 Step G: Estimation of costs for storage and documentation and comparison of these costs with available or expected funding

When the desired storage conditions have been designed, it is recommended to make an estimation of the costs for storage and documentation, which is as detailed as possible, and to compare these estimated costs with the available or expected funding.

If the costs are excessive, it is necessary to consider changes in the study programme, e.g. by reassessing the need for further analysis or the duration of storage, or to reconsider the need for further analysis altogether. The consequences should be discussed with the client, in order to decide what is the optimal solution.

15 Test report

Test reports from analysis of soils after storage should include a description of the conditions of storage used.

Annex A
(normative)

Storage for soil samples

Table A.1 — Maximum storage times for soils at different conditions — Physical and chemical test objectives

Test objective	Reference to International Standard	Dried ambient temperature	Dried 4 °C	Wet 4 °C[a]	Wet –18 °C	Wet –80 °C	Reference to subclause of this International Standard
Soil characteristics							
Electrical conductivity	ISO 11265	3 y	3 y	1 w	10 y[b]	10 y[b]	11.2.1
pH	ISO 10390	3 y	3 y	1 w	10 y[b]	10 y[b]	11.2.2
Inorganic parameters							
Heavy metals[c]							11.3.1
— Total amount	ISO 11466	30 y[b]	30 y[b]	6 m[b]	10 y[b]	30 y[b]	
— Mobile amount		1 y	3 y	1 m	ne	ne	
Hg (volatile)		—	—	4 d	ne	ne	11.3.1
Cr(VI)	EN 15192	ne	ne	30 d	ne	ne	
Macro-nutrients							11.3.1-3
Total:							
— P, K, Ca, Mg	ISO 11261	30 y	30 y	ne	10 y[b]	30 y[b]	
— N	ISO 13878	30 y	30 y	1 m	10 y[b]	30 y[b]	
Available							
— P	ISO 11263	3 y	1 m	1 w	10 y	30 y[b]	
— K, Ca, Mg	ISO 14255	3 y	1 m	1 w	10 y	10 y[b]	
— N_{min}	ISO 14256	—	—	1 w	5 w	10 y[b]	
Anions							11.3.4
— F, Cl, Br		3 y	3 y	1 m	10 y[b]	10 y[b]	
— SO_4	ISO 11048	3 y	3 y	1 m	10 y[b]	10 y[b]	
Organic parameters							
Chlorophenols	ISO 14154	—	—	4 d[d]	ne	ne	
EOX		ne	ne	1 w 1 m[e]	ne	ne	
Humus, C_{org}	ISO 10694	3 y	3 y	1m	3 y	10 y[b]	
Halogenated hydrocarbons, highly volatile	ISO 10301	—	—	4 d 1 m[f]	4 d 1 m[f]	ne	11.3.5
Hydrocarbons, C10-C40	ISO 16703	ne	ne	1 w 1 m[e]	1 m	ne	11.3.6
Organochlorine pesticides and PCB	ISO 10382	—	—	1 m	6 m	ne	11.3.6

Table A.1 *(continued)*

Test objective	Reference to International Standard	Dried ambient temperature	Dried 4 °C	Wet 4 °C[a]	Wet −18 °C	Wet −80 °C	Reference to subclause of this International Standard
Organonitrogen pesticides		—	—	1 w	ne	ne	11.3.6
Organophosphorous pesticides		—	—	1 w	ne	ne	11.3.6
PAH	ISO 13877	—	—	2 w[g] 1 m[f]	6 m	ne	11.3.5 11.3.6
PCDD/PCDF		3 to 6 m	3 to 6 m	1 y	10 y	10 y	
VOC	ISO 22155 ISO 15009	—	—	4 d 1 m[f]	1 w 1 m[f]	ne	11.3.5
Organic and inorganic test objectives							
Leaching							
— Release of pollutants		3 y	3 y	1 m	1 y[b]	10 y[b]	
— Column test		3 y	3 y	1 m	1 y[b]	10 y[b]	
— DOM		1 y	3 y	1 m	3 y	10 y[b]	

[a] The short storage times for refrigerated wet samples reflect the risk for biological activity under these conditions.

[b] Expert judgement: not experimentally proved.

[c] In the case of Hg, these recommendations refer only to the non-volatile Hg-compounds.

[d] ISO 14154 recommends freezing the samples if storage during more than 2 days is required.

[e] After chemical drying with Na_2SO_4, as described in ISO 14507.

[f] Storage time in methanol.

[g] It has been shown that naphthalene sometimes degrades within the given period. Therefore, samples in which naphthalene has to be analysed should be stabilized within 4 days by adding solvent or by chemical drying.

Symbols

ne no experience

— no storage possible

d days

w week(s)

m month(s)

y year(s)

Abbreviations

DOM: dissolved organic matter

EOX: extractable organic halogenic compounds

PAH: polyaromatic hydrocarbons

PCB: polychlorinated biphenyls

PCDD/PCDF: polychlorinated dibenzodioxins/polychlorinated dibenzofurans

VOC: volatile organic compounds

Table A.2 — Maximum storage times for soils at different conditions — biological test objectives

Test objective	Reference to standard	Dried Ambient temperature	Dried 4 °C	Wet 4 °C[a]	Wet –18 °C	Wet –80 °C	Reference to subclause of this International Standard
Ammonium oxidation	ISO 15685	—	—	3 m	1 y	ne	11.4.1
Biodegradation							11.4.2
— Aerobic	ISO 11266	—	—	3 m	ne	ne	
— Anaerobic	ISO 15473	—	—	3 m[b]	ne	ne	
Biomass							11.4.1
— Substrate-induced Respiration method	ISO 14240-1	—	—	3 m	1 y	ne	
— Fumigation extraction method	ISO 14240-2	—	—	3 m	1 y	ne	
Dehydrogenase activity	ISO 23753-1 ISO 23753-2	—	—	3 m	1 y	ne	11.4.1
DNA[c]		—	—	3 m[d]	1 to 2 y	ne	11.4.1
Microbial soil respiration	ISO 16072	—	—	3 m	1 y	ne	11.4.1
Nitrogen mineralization	ISO 14238	—	—	3 m	1 y	ne	11.4.1
Plant test	ISO 11268-1, ISO 11268-2 ISO 22030	ne	ne	3 m	1 y	ne	11.4.3
PLFA[c]		—	—	3 m[d]	1 to 2 y	ne	11.4.1
RNA[e]		—	—	ne	6 to 8 m	2 to 4y	11.4.1
Soil fauna							11.4.3
— Earthworm	ISO 11268-1, ISO 11268-2	—	—	3 m	1 y	ne	
— Collembola	ISO 11267	—	—	3 m	1 y	ne	
— Enchytraeids	ISO 16387	—	—	3 m	1 y	ne	
— Insect larvae	ISO 20963	—	—	3 m	1 y	ne	
— Land snails	ISO 15952	—	—	3 m	1 y	ne	
Soil respiration curves	ISO 17155	—	—	3 m	1 y	ne	11.4.1
Soil used as a dilution or reference medium	ISO 15799	un	un	ne	ne	ne	11.4.3

[a] The short storage times for refrigerated wet samples reflect the risk for biological activity at these conditions.

[b] Only for determination of anaerobic degradation of chemicals in aerobic soils; no statements for storage of anaerobic soils are given.

[c] The soils should 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 extract can be stored at –20 °C for several months prior to further separation and analysis with GC/MS.

[d] Expert judgement: not experimentally proven.

[e] Shock freezing in liquid nitrogen is recommended before storage at – 20 °C or – 80 °C.

Symbols

ne no experience
— no storage possible
d days
w week(s)
m month(s)
y year(s)
un unlimited

Abbreviations

PFLA: phospholipid fatty acids

Bibliography

[1] FIERER, N. and SCHIMEL, J.P. (2002): Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry* **34**: pp. 777-787

[2] HAYNES, R.J. and BEARE, M:H. (1996): *Aggregation and organic matter storage in meso-thermal, humid soils*. In: Carter, M..R., Steward, B.A. (Eds) Soil Structure and Organic Matter Storage. CRC/Lewis Publishers, Boca Raton, pp. 213-262

[3] HOUBA, V.J.G. and NOVOZAMSKY, I. Influence of storage time and temperature of air-dried soils on pH and extractable nutrients using 0,01 M $CaCl_2$. *Fresenius J. Anal.Chem.* 1998, **360**, pp. 362-365

[4] LICKFETT, T., MERKEL, D., HORSTEN, D., von, PRZEMECK, E. und LUCKE, W. Konservierung von Nmin-Proben durch Mikrowellentrocknung, *Agribiol. Res.* **49**, 1996 (in German)

[5] LUND, V. and GOKSØR, J. (1980): Effects of Water Fluctuations on Microbial Mass and Activity in Soil. *Microbial Ecol.* **6**: pp. 115-123

[6] MEHLICH, A (1984) Mehlich 3 soil test extractant: A modification of Mehlich 2. *Communications of Soil Science and Plant Analysis* **15**, pp. 1409-1416

[7] WEINFURTNER, K., DREHER, P., HUND-RINKE, K., SCHEID, S. and SIMON, M. (2002): *Methodische Weiterentwicklung der Probenrichtlinie für Böden im Rahmen der Umweltprobenbank*. Abschlussbericht UBA, FKZ 301 02 006 (unpublished report)

[8] ZBÍRAL, J., NEMEC, P. *The influence of the sample treatment on the determination of the nitrate and ammonium forms of nitrogen*. Bulletin of the Laboratory Division, 1998, volume II, issue 1 (in Czech)

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

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

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

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

[13] ISO 11464, *Soil quality — Pretreatment of samples for physico-chemical analyses*

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