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Soil quality — Pretreatment of samples by freeze-drying for subsequent analysis

Qualité du sol — Prétraitement des échantillons par lyophilisation pour analyse subséquente

Reference number
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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

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 16720 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical methods and soil characteristics*.

Soil quality — Pretreatment of samples by freeze-drying for subsequent analysis

1 Scope

This International Standard specifies a method for pretreatment of soil samples by freeze-drying for subsequent analysis.

This International Standard is applicable to soil samples for subsequent determination of elements or organic compounds recognized as non-volatile under freeze-drying conditions. Generally, this International Standard can also be applied to samples from sludges and sediments.

This method is also applicable as a first step for the determination of dry matter (or water) content, for instance in the case of samples with high water content.

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 11464, *Soil quality — Pretreatment of samples for physico-chemical analyses*

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

3 Principle and general requirements

Before freeze-drying, samples shall be cooled below their eutectic points. These are rarely known, but freezing the samples below –40 °C is usually sufficient.

During freeze-drying, water is removed from the sample under vacuum conditions by direct conversion from ice to vapour (sublimation) which is collected in a condenser where it releases its heat energy and turns again into ice. Generally a temperature below –50 °C inside the condenser is suitable for usual applications.

Sublimation requires heat in order to take place within an acceptable range of time. When the frozen sample containers are connected to an external inlet manifold (see 5.1.2), heat energy is supplied by the laboratory atmosphere. In case of a drying chamber equipped with temperature-controlled carriers (see 5.1.1), this energy is provided by heating the carriers.

Vacuum conditions shall allow the generation of vapour pressure difference needed for sublimation and the continuous evacuation of water vapour from the sample. Throughout the drying process, the pressure inside the apparatus and the temperature of the sample(s) shall be such as to avoid sample thawing and loss of any compound of interest. This last point may be difficult to achieve due to the large range of possible energy levels of the bonds between compounds of interest and the solid phase. For compounds known as non-volatile, a final temperature of the sample between –20 °C to –25 °C is suitable. Other final temperatures may be needed for compounds recognized as more volatile. In all cases, the final step shall be as short as possible.

A sample in thin layer form is required for suitable freeze-drying. Whatever the kind of sample container, the thickness of the sample should not exceed 2 cm. If the representative amount exceeds a thickness of 2 cm, multiple drying may be applied.

The quantity of sample treated depends on the representativeness, the dry matter content and the number of subsequent determinations. Sample containers for freeze-drying are selected accordingly.

NOTE Soil samples often need to be dried before analysis. In the case of very humid or clayey soils, the technique of freeze-drying compared with air or oven drying at temperatures < 40 °C in accordance with ISO 11465 has the advantage of a quicker drying process and provision of a dried sample that can be reduced in size more easily.

4 Interferences

Substances may volatilize during freeze-drying and may partly be released to the ice condensate or be found in the gas released to the vacuum pump. If compounds lost due to valatilization are amongst those included in the list of parameters to be determined subsequently, analysis results are then directly affected. In contrast, mass losses only affect the dry matter content and this effect is generally negligible.

NOTE For example, ammonia nitrogen is lost during freeze-drying of sludges, the intensity of loss depending on the dry matter content. Therefore determination of ammonia nitrogen content should be avoided after freeze-drying, at least for liquid sludges, but in such a case the dry matter content is not significantly affected.

In the final step of freeze-drying, samples become pulverulent and turbulences induced by the vacuum pump may carry away smaller solid particles. Samples can be protected by means of a wire mesh.

Achieving total dryness is difficult and may consume too much time, especially when several samples are treated at the same time. In these case, a level of dryness should be achieved which merely allows the samples to be handled, ground and homogenized, and residual water content determined afterwards on a sub-sample.

If not free, water may interact more or less strongly with the solid phase, leading to residual water contents depending on the selected drying method. For a standard determination of dry matter or water content, drying at 105 °C in accordance with ISO 11465, is required after freeze-drying.

5 Apparatus

5.1 Freeze-drying apparatus, composed of a drying device (5.1.1 or 5.1.2) connected to a condensing chamber (condenser), a vacuum system, a cooling system and the appropriate controls.

5.1.1 Thermally isolated drying chamber equipped with temperature controlled carriers.

The temperature of the samples, measured by inserted probes, is controlled by heating or cooling the carriers during freeze-drying. Generally, samples can be frozen on the carriers prior to freeze-drying.

5.1.2 External inlet manifold to which the frozen sample containers (generally flasks, but a few apparatus allow the use of trays instead of flasks) are connected.

Heat energy is supplied by exchange with the laboratory atmosphere.

5.2 Suitable sample containers for freeze-drying, trays or flasks, forms and sizes are depending on the type of apparatus selected or available and on the quantities of sample treated.

5.3 Freezer for pre-cooling of samples, if possible with contact freezing facilities, minimum temperature –35 °C, explosion-protected.

Instructions of the manufacturer shall be followed.

5.4 Pre-cooling equipment with a rotating system and a cooling bath containing ethanol at –40 °C.

Instructions of the manufacturer shall be followed.

5.5 Suitable storage containers for freeze-dried material, e.g. brown glass bottles.

6 Procedure

6.1 Pre-cooling of samples

Transfer a representative amount of the sample into a suitable container (5.2). The thickness of the layer in the plate should not exceed 2 cm. Multiple drying is allowed if the thickness of the representative amount exceeds 2 cm.

NOTE Layers with thickness < 2 cm accelerate the freezing and drying process.

Transfer the container with the sample into the freezer (5.3) or drying chamber (5.1.1), taking care to obtain a horizontal alignment. If necessary, stick a temperature probe into the sample (see 5.1.1). Follow the instructions of the manufacturer of the freeze-drying device. For apparatus operating with flasks (5.1.2), the thickness of the layer on the flask wall shall be as thin as possible. Use of a rotating system (5.4) is recommended.

If determination of dry matter content is required, weigh the empty container and record the mass (m_1). Then weigh the container with the sample and record the mass (m_2) (see 6.5).

6.2 Freeze-drying

When using device 5.1.1, allow the drying chamber to cool (if not already achieved with pre-cooling).

Pre-cool the condenser in accordance with instructions of the manufacturer.

When using device 5.1.1, place the tray(s) with the frozen sample(s) on the carrier(s) of the drying chamber. If necessary connect the temperature probe(s). Close the chamber door tightly. Start the heating and temperature regulation of the carriers in accordance with the instructions of the manufacturer, so that the sample will not thaw.

When using device 5.1.2, connect the flask(s) to the inlet manifold. Start the vacuum pump and wait until the vacuum is stable and the pressure is between 37 Pa and 63 Pa, so that the sample(s) will not thaw.

NOTE A pressure of 37 Pa equals an ice temperature of approximately –30 °C. A pressure of 63 Pa equals an ice temperature of approx. –25 °C.

6.3 End of drying process

The detection of the end of the drying process is crucial. The treatment of several different samples in a single batch increases the difficulty.

The end of the process can be observed using the following parameters:

- specific instructions of the manufacturer;
- pressure rise is minimal, or no further change in pressure occurs;

NOTE Compared to the empty system, the pressure does not increase when all the ice has been sublimed.

- final temperature of the condenser approaches its minimum value and remains constant (within 2 h, for example);
- temperature of the dried product starts to rise to ambient (5.1.2) or to the maximum temperature selected for the carriers (5.1.1) (see Clause 3).

Following freeze-drying, carefully open the appropriate valve to return the drying chamber to atmospheric pressure, stop the vacuum pump and condenser cooling system.

6.4 Dried-sample treatment and handling

Due to the tendency of freeze-dried samples to produce dust particles, hygienic requirements shall be observed while preparing the sample.

If dry matter content (mass fraction) is required, weigh the container with the dried sample and record the mass (m_3) (see 6.5).

For the determination of inorganic substances, prepare the sample in accordance with ISO 11464 prior to analysis. For the determination of organic substances, sieve the sample to obtain particle size 2 mm.

For further analytical treatment, transfer the prepared sample into a container (5.5).

Depending on the level of dryness, the dried sample may be hygroscopic and may absorb atmospheric water. In such a case special care shall be taken, because the residual water content just after freeze-drying (m_3) may be significantly different to the water content after grinding and sieving the sample. After weighing the freeze dried sample, take a representative sub-sample and determine the residual water content in accordance with ISO 11465. Repeat this determination on the prepared sample, record the difference between the two values and if necessary take it into account in the calculation of the results of subsequent analysis relative to a dry sample or a fresh sample.

6.5 Determination of dry matter content

The sample obtained after freeze-drying shall be considered as a partially dried sample.

The “partially dried” dry matter content (w_{pd}) is calculated according to Equation (1):

$$w_{\text{pd}} = \frac{(m_3 - m_1) \times f}{(m_2 - m_1)} \tag{1}$$

where

w_{pd} is the “partially dried” matter content, in percent (mass fraction);

m_1 is the mass of the empty container, in grams;

m_2 is the mass of the container with undried sample, in grams;

m_3 is the mass of the container with dried sample, in grams;

f is the factor for calculation; $f = 100\ \%$.

In order to express the result relative to a sample dried at 105 °C, the residual water content (w_{r}) shall be determined on the prepared sample (6.4) in accordance with ISO 11465. Dry matter content (w_{dm}) is then calculated according to Equation (2):

$$w_{\text{dm}} = \frac{(m_3 - m_1) \times f}{(m_2 - m_1)} \times \frac{f}{w_{\text{r}} + f} \tag{2}$$

where

w_{dm} is the dry matter content, in percent;

w_{r} is the residual water content, in percent.

If water re-absorption is suspected, follow the procedure given in 6.4.