INTERNATIONAL STANDARD

ISO 5667-15

Second edition
2009-08-15

Water quality — Sampling —

Part 15: Guidance on the preservation and handling of sludge and sediment samples

Qualité de l'eau — Échantillonnage —

Partie 15: Lignes directrices pour la conservation et le traitement des échantillons de boues et de sédiments

Reference number
ISO 5667-15:2009(E)

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Published in Switzerland

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

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

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 5667-15 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 6, *Sampling (general methods)*.

This second edition cancels and replaces the first edition (ISO 5667-15:1999), which has been technically revised.

ISO 5667 consists of the following parts, under the general title *Water quality — Sampling*:

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

— *Part 3: Guidance on the preservation and handling of water samples*

— *Part 4: Guidance on sampling from lakes, natural and man-made*

— *Part 5: Guidance on sampling of drinking water from treatment works and piped distribution systems*

— *Part 6: Guidance on sampling of rivers and streams*

— *Part 7: Guidance on sampling of water and steam in boiler plants*

— *Part 8: Guidance on the sampling of wet deposition*

— *Part 9: Guidance on sampling from marine waters*

— *Part 10: Guidance on sampling of waste waters*

— *Part 11: Guidance on sampling of groundwaters*

— *Part 12: Guidance on sampling of bottom sediments*

— *Part 13: Guidance on sampling of sludges from sewage and water-treatment works*[1)]

1) In preparation. (Revision of ISO 5667-13:1997)

— *Part 14: Guidance on quality assurance of environmental water sampling and handling*

— *Part 15: Guidance on the preservation and handling of sludge and sediment samples*

— *Part 16: Guidance on biotesting of samples*

— *Part 17: Guidance on sampling of bulk suspended solids*

— *Part 19: Guidance on sampling of marine sediments*

— *Part 20: Guidance on the use of sampling data for decision making — Compliance with thresholds and classification systems*

— *Part 21: Guidance on sampling of drinking water distributed by tankers or means other than distribution pipes*

— *Part 22: Guidance on the design and installation of groundwater monitoring points*

— *Part 23: Determination of priority pollutants in surface water using passive sampling*

This part of ISO 5667 may be used in conjunction with the other parts available within the ISO 5667 series.

Water quality — Sampling —

Part 15: Guidance on the preservation and handling of sludge and sediment samples

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This part of ISO 5667 provides guidance on procedures for the preservation, handling and storage of samples of sewage and waterworks sludge, suspended matter, saltwater sediments and freshwater sediments, until chemical, physical, radiochemical and/or biological examination can be undertaken in the laboratory.

The procedures in this part of ISO 5667 are only applicable to wet samples of sludge, sediment and suspended matter.

NOTE Samples of sludge, sediment and suspended matter that are dried or freeze-dried behave similarly to dried soils. For guidance on long- and short-term storage of (freeze) dried samples, see ISO 18512. For guidance on freeze-drying, see ISO 16720.

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 3696, *Water for analytical laboratory use — Specification and test methods*

3 Terms and definitions

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

3.1
sample preservation
any procedure used to stabilize a sample in such a way that the properties under examination are maintained stable from the collection step until preparation for analysis

[ISO 11074:2005, 4.4.20]

3.2
sample storage
process, and the result, of keeping a sample available under predefined conditions for a (usually) specified time interval between collection and further treatment of a sample

NOTE Adapted from ISO 11074:2005, 4.4.22.

3.3
storage duration
period of time between sample collection and start of the analysis of the sample in the laboratory, for a sample stored under pre-defined conditions

4 Reagents

WARNING — Sampling personnel should be warned of potential dangers and appropriate safety procedures should be available. Beware of formaldehyde vapours. Do not store large numbers of samples in small working areas.

All reagents used should be of at least analytical reagent grade.

4.1 Deionized water, Grade 3 quality as specified in ISO 3696.

4.2 Sodium sulfate, Na_2SO_4, monohydrate.

Heat the sodium sulfate before use for at least 6 h at (500 ± 10) °C. Store in an desiccator after heating.

4.3 Zinc acetate, $(CH_3COO)_2Zn{\cdot}2H_2O$ (10 % mass fraction).

4.4 Methanol, CH_3OH.

4.5 Ethanol, C_2H_5OH (volume fraction of 96 %).

4.6 Sodium tetraborate ($Na_2B_4O_7{\cdot}10H_2O$), **sodium phosphate** ($Na_4P_2O_7{\cdot}10H_2O$) or **hexamethylene-tetramine** [$(CH_2)_6N_4$].

4.7 Formaldehyde solution, CH_2O (volume fraction of 3,7 %).

Add 37 % formaldehyde neutralized to pH 7 with sodium tetraborate, sodium phosphate or hexamethylenetetramine (100 g/l formalin solution) to give a final solution of 3,7 % formaldehyde (corresponding to a 1 to 10 dilution of formalin solution).

NOTE 37 % formaldehyde is 100 % formalin.

5 Preservation of samples

5.1 General considerations

Sample handling is specific for each determination to be conducted. Manipulation of samples is often required to yield consistent material for toxicity testing and laboratory experiments. Homogenization, by mixing or sieving, dilution to obtain a suitable concentration and addition of chemical preservatives all complicate interpretations of *in situ* comparisons.

The purpose of preservation is to retain the integrity of the collected material as it was on site in relation to the parameters to be analysed. Analytes might biodegrade, volatilize, oxidize, be reduced or photolyse during storage. Therefore, careful consideration should be given to these processes and the storage conditions needed to avoid such alterations.

The need to preserve sludge, sediment and suspended matter begins immediately after a sample has been taken. The most critical changes to the sample can occur in the first few hours after sampling. Therefore, where possible, preservation steps should be taken immediately upon sample collection.

The choice of preservation technique depends mainly on the objective of the sample collection and the analysis being determined. It is important to understand the effects that preservation and storage can have on the sample quality and the analysis results.

No recommendations can be given for a universal preservation or storage method. A preservation method used for one group of parameters can interfere with the analysis of other groups of parameters. To overcome this problem, a number of sub-samples should be collected; each sub-sample should be preserved using a different method such that the full range of required analyses is represented.

5.2 Chemical examination

Chemical analysis can be performed to determine the nature and amounts of the substances that have become absorbed or adsorbed by sludge, sediment and suspended matter.

Partition of chemical components between the solid phase and the water phase is influenced by several factors, such as particle size, amount of organic matter, pH, redox potential and salinity. The study of such attributes can be a sampling objective. Therefore, the preservation needs for the analytical methods to be employed should be taken into account (see Table 1). The guidance given in this part of ISO 5667 is relevant to the determination of components in the sum of the separate phases of a sludge or sediment, unless otherwise indicated.

Preservation of samples by fast-freezing can cause mobilization of contaminants by cellular disruption, whereas not stabilizing samples can permit continued microbial transformation of critical parameters of interest. In addition to biodegradation of organics, volatilization is a principal mechanism of loss of volatile compounds during sample handling. Microbial activity can be responsible for changes in the nitrate-nitrite-ammonia content, for decrease in biochemical oxygen demand, or for reducing sulfate to sulfide. Anoxic samples require appropriate preservation techniques such as oxygen exclusion during sample handling. Drying, freezing and freeze-drying of anoxic samples alter the binding sites of, for example, heavy metals, making more differentiated investigation of binding forms virtually impossible.

5.3 Physical examination

The structure, texture and, for sediments, the layer formation should be determined.

NOTE Sediment matrix changes are obvious if rapid drainage of pore water occurs.

The importance of sludge or sediment integrity to the investigation objectives should be evaluated as it can influence the preservation and handling techniques. In general, any disturbance of the samples should be minimized. Where the physical structure of the material sampled is important for the measurement of parameters (e.g. resistance to filtration), agitation and vibration during transport should be reduced to a minimum. Fast-freezing of the sludge and sediments may be appropriate. In some cases, thermal techniques should be avoided as they strongly modify sludge structure, thus affecting physical characteristics (e.g. de-waterability, settleability, flowability).

Samples should be stored and preserved in accordance with the conditions given in Table 1.

5.4 Radiochemical examination

Some sample sites can have measurable radiochemical activity in the soil or air. Some items of domestic equipment within the laboratory can also be a source of radioactive material. Infection of the sample by its environment should therefore be avoided, especially if the sample activity is likely to be very low.

Samples should be stored and preserved in accordance with the conditions given in Table 2.

5.5 Biological examination

Biological studies include toxicological, ecotoxicological and ecological examinations. The same factors mentioned in relation to chemical examination (see 5.2) can alter the bioavailability and toxicity of compounds.

The assessment of sludge contamination by laboratory bioassay testing requires different preservation techniques in comparison to ecological or microbial investigation. An ecological investigation generally involves classifying the species and numbers of flora and/or fauna present on and in fixed sludge or sediments.

Microbial activity may also be used to characterize samples and can only be determined without fixation.

Any large individuals of macrofauna should be removed from the samples immediately after collecting samples taken for the chemical, physical, radiochemical and/or biological examinations.

Samples should be stored and preserved in accordance with the conditions given in Tables 1 and 3.

6 Safety precautions

6.1 Staff protection

Health and safety precautions should be observed at all times when sampling potentially hazardous sludge, sediments or suspended matter.

Human exposure to pathogenic organisms or pollutants should be avoided by using appropriate protective equipment such as respiratory protective masks, safety glasses and protective gloves. The hazard due to pathogenic organisms can be very high. It is vital that all sampling personnel should receive thorough training and be provided with appropriate medical inoculations.

Degradation of sludge produces methane, which presents a risk of fire and explosion if a source of ignition is present. Containers should be appropriately wrapped to minimize the fragmentation of the containers if an explosion occurs.

If sludge samples are to be taken in locations where there is restricted ventilation, staff should take safety precautions to protect themselves against sulfide, carbon dioxide and methane.

6.2 Sample protection

When sampling, transporting and utilizing sludge, care should be taken to prevent a build-up of gas pressure in the sample container. Manual release of pressure during and after transport may be necessary if prolonged storage is required.

Samples collected for the analysis of volatile organic or sulfide compounds should not be homogenized because many of these compounds could be lost while compositing.

7 Containers

Sample containers should be made of a material appropriate for preserving the natural properties of both the sample and the expected range of contaminants. Suitable types of container for each analyte to be measured are given in Tables 1, 2 and 3.

If the samples are to be frozen, suitable material such as polyethylene or polytetrafluoroethylene (PTFE) should be used to minimize the risk of breakage.

Careful consideration should also be given to the suitability of the container for cleaning/decontamination or disposal and appropriate action taken. Recommendations for the preparation of containers are given in Annex A.

The choice of sample container is of major importance and ISO 5667-1 provides guidance on this subject.

In 5.3 of ISO 5667-14:1998, guidance is given on measuring the contamination impact of the container. The analyte level in the blank should be negligible compared to the analyte level to be measured in the sample.

NOTE Regular container volumes are 500 g to 1 000 g.

8 Sample collection

Samples should be collected in sufficient volumes to allow:

a) separate sub-samples to be preserved for each type of analysis or examination to be undertaken;

b) repeat the analysis in the event of error checking or the routine quality control requirements of duplicate analysis;

c) prepare time-dependent composites; for example, a daily aliquot of sewage works sludge (preserved as appropriate) may be retained to produce a composite for monthly analysis.

For sludge samples, it is recommended that the container is filled to a maximum of 80 % of its capacity, especially if biological activity is expected, in order to reduce the risk of overpressurization and explosion.

If analysis of volatile compounds is required, containers should be completely filled with sample sediment from the first grab, prior to sample homogenization. No headspace should remain in either container.

If the sample is to be frozen, enough headspace should be allowed for expansion to take place.

Where samples are collected for the purpose of microscopic examination, for example of activated sludges, it is recommended to fill the container to no more than 5 % of its capacity to ensure an oxygen supply to the sludge prior to examination.

The temperature of the sample, especially of sludge samples, can influence the properties of the sample. Therefore, the initial temperature of the sludge samples should be measured on site and recorded.

9 Identification of samples

Container labels should withstand wetting, drying and freezing without detaching or becoming illegible. The labelling system should be waterproof to allow use in the field.

The exact information given in the sampling report and on the sample labels will depend on the objectives of the particular measurement programme. In all cases, an indelible label should be secured to the sample container.

For each sample, the following information should be provided as a minimum:

— a unique identifier, traceable to the date, time and location of sampling;

— a description and disposition of sample;

— the name of the individual sampler;

— details of preservation used;

— details of sample storage used;

— any information regarding integrity and manipulation of the sample;

— other information as necessary.

The unique identifier should be on the label of the sample container. The remaining information can be provided in the sample report.

10 Transport of samples

During transportation, samples should be stored in a cooling device capable of maintaining a temperature between 2 °C and 8 °C. It is recognized that not all sample locations allow for immediate storage under such conditions for practical and safety reasons. Where this arises appropriate facts should be recorded in the sample report.

However, samples that need to be frozen for preservation purposes (see Tables 1, 2 and 3) should be frozen on site and transported at a temperature below −18 °C.

The temperature of the cooling device, refrigerator and/or freezing device should be recorded and the information provided in the sample report.

NOTE In circumstances where the temperature of the cooling device is outside this range for a period of time during transport (e.g. when opening the cooling device), the laboratory and data user will need to determine what effect this can have on the samples and/or the results of the analyses.

If physical sample integrity is important, agitation and vibration during transport should be reduced to a minimum (see also 5.3).

11 Reception of samples

Laboratory staff should receive and check relevant information on preservation and transport conditions of the sample. All information regarding sample manipulation, handling and storage should therefore be recorded and reported with the results of testing.

In all cases, and especially when a “chain of custody” process needs to be established, the number of sample containers received in the laboratory should be verified against the number of sample containers submitted, together with the condition of the containers and the integrity of any seals applied in the field.

12 Sample storage

The storage duration of samples of sludge, sediment and suspended matter within the laboratory is specific to the analyte(s) to be analysed. Samples should be stored no longer than the maximum storage duration times given in Tables 1, 2 and 3, with the exception of wet sediments that may be stored for longer periods when samples are preserved using a nitrogen vapour freezer.

NOTE 1 For guidance on long-term storage of wet sediment samples using nitrogen vapour freezers, see Annex B.

NOTE 2 For further information on long- and short-term storage of dried samples, see ISO 18512.

Be aware of the fact that the cooling conditions within the laboratory (1 °C to 5 °C) are different from the cooling conditions during transport (2 °C to 8 °C).

Table 1 — Type of container, preservation and storage conditions for different analytes to be studied in sediments, suspended matter and sludges

Analyte to be studied	Type of container [a]	Minimum sample size [b] g	Preservation and storage conditions	Maximum storage duration [c]	Comments
Acidity	P or G	50	1 °C to 5 °C, dark and airtight	14 days	
Alkalinity	P or G	50	1 °C to 5 °C, dark and airtight	14 days	
Ammoniacal nitrogen	P or G	50	1 °C to 5 °C, dark and airtight	24 h	Sludge parameter
Anions (Cl, Br, F and SO_4)	P or G	50	1 °C to 5 °C, dark and airtight	1 month	
Adsorbable organically bound halogens (AOX)	P or G	50	1 °C to 5 °C, dark and airtight	7 days	
Biodegradation	P or G	50	1 °C to 5 °C, dark and airtight	24 h	
Biochemical (biological) oxygen demand (BOD)	P or G	50	1 °C to 5 °C, dark and airtight	24 h	
			< -18 °C	1 month	
Capillary suction time (CST)	P or metal	1 000	1 °C to 5 °C, airtight	24 h	Sludge parameter
Conductivity	P or G	50	1 °C to 5 °C, dark and airtight	24 h	
Chromium VI	P or G	50	1 °C to 5 °C, dark and airtight	24 h (sludge) 2 days (sediment)	
Cyanides	P	50	< -18 °C	1 month	
	G	50	1 °C to 5 °C, dark and airtight	4 days	
Dry matter (dry mass)	P or G	50	1 °C to 5 °C, airtight	7 days	For determination of dry weight at sub-sampling, the storage duration is unlimited
Extractable organic halogens (EOX)	see “Adsorbable organically bound halogens (AOX)”				
Kjeldahl nitrogen	P or G	50	1 °C to 5 °C, dark and airtight	24 h (sludge) 7 days (sediment)	
Mercury (non-volatile)	P or G	50	1 °C to 5 °C, dark and airtight	1 month	
			< -18 °C, dark and airtight	1 month	
Mercury (volatile)	P or G	50	1 °C to 5 °C, dark and airtight	4 days	

Table 1 (*continued*)

Analyte to be studied	Type of container [a]	Minimum sample size [b] g	Preservation and storage conditions	Maximum storage duration [c]	Comments
Metals	P or G	50	1 °C to 5 °C, dark and airtight	1 month	
			< −18 °C, dark and airtight	6 months	
	P or G		Dry at approx. 60 °C and store at ambient temperature; dark and airtight	6 months	Not allowed for mercury
Microscopic analysis	G	10	1 °C to 5 °C	24 h	
Mineral oil (hydrocarbons C10-C40)	G	100	1 °C to 5 °C, dark and airtight	1 month	
	P		< −18 °C	6 months	
	G		Add sodium sulfate (4.2): 25 g on 50 g of sample	6 months	
Nitrate	P or G	50	1 °C to 5 °C, dark and airtight	24 h (sludge) 7 days (sediment)	
Nitrification	P or G	50	1 °C to 5 °C, dark and airtight	24 h	
Nitrite	P or G	50	1 °C to 5 °C, dark and airtight	Preferably analysis on site, but at least within 24 h	
Oil and grease	G	100	1 °C to 5 °C, dark and airtight	1 month	
	P		< −18 °C	6 months	
	G		Add sodium sulfate (4.2): 25 g on 50 g of sample	6 months	
Organonitrogen and organophosphorous pesticides	G with PFTE-lined cap	50 per group	Extract and store at 1 °C to 5 °C, dark and airtight	1 month	
Organotin compounds	G	50	1 °C to 5 °C, dark and airtight	7 days	
			< −18 °C, dark and airtight	6 month	
Orthophosphate	P or G	50	1 °C to 5 °C, dark and airtight	24 h (sludge) 2 days (sediment)	
Particle size distribution	P or G	1 000 (sludge) 100 (sediment)	1 °C to 5 °C, dark and airtight	24 h (sludge) 1 month (sediment)	No preservation allowed
PCB, PAH, chloropesticides	G with PTFE-lined cap	50 per group	1 °C to 5 °C, dark and airtight	1 month	

Table 1 (*continued*)

Analyte to be studied	Type of container [a]	Minimum sample size [b] g	Preservation and storage conditions	Maximum storage duration [c]	Comments
pH (in the field)	Sampling device	50	Wet undisturbed	None	Determine on site
pH (at the laboratory)	P or G	50	1 °C to 5 °C, dark and airtight	24 h	
Phosphorus (total)	P or G	50	1 °C to 5 °C, dark and airtight	24 h (sludge) 1 month (sediment)	
Respiration	P or G	50	1 °C to 5 °C, dark and airtight	24 h	
Semi- and non-volatile organic compounds	G with PTFE-lined cap	50 per group	Extract and store at 1 °C to 5 °C, dark and airtight	1 month	
			Extract and store at < -18 °C, dark and airtight	6 months	
Settleability/ thickenability	P or metal	5 000	1 °C to 5 °C, airtight	24 h	Sludge parameter
Specific resistance to filtration	P or metal	2 500	1 °C to 5 °C, airtight	24 h	Sludge parameter
Sulfide	P or G	50	pH $> 10,5$; 1 °C to 5 °C; dark, airtight and anoxic	24 h	
			Add 5 ml of 10 % zinc acetate	7 days	
Total organic carbon (TOC)/ inorganic carbon (IC)	G with PTFE-lined cap	25	1 °C to 5 °C, dark and airtight	1 month	
			< -18 °C, dark and airtight	6 months	
Volatile organics	G with PTFE-lined cap	50	1 °C to 5 °C, dark and airtight	4 days	
			Extract with methanol and store at 1 °C to 5 °C, dark and airtight	1 month	
			Extract with methanol and store at < -18 °C, dark and airtight	6 month	

[a] P = Plastics, e.g. PE (polyethylene), PTFE (polytetrafluoroethylene), PVC [poly(vinyl chloride)], PET [poly(ethylene terephthalate)].

G = Glass.

BG = Borosilicate glass.

[b] Minimum field sample size for the determination of the specific analyte, based on wet material. In the case where more than one analyte is analysed on the same field sample, a sample mass less than the sum of the masses may be sufficient.

[c] Including duration of transport.

Table 2 — Techniques generally suitable for the preservation of samples for radiochemical analysis

Analyte to be studied	Type of container [a]	Minimum sample size [b] g	Preservation and storage conditions	Maximum storage duration [c]	Comments
Alpha activity	P	100	1 °C to 5 °C	1 month	
Beta activity (except radio-iodine)	P	100	1 °C to 5 °C	1 month	
Gamma activity	P	100	1 °C to 5 °C	2 days	
Radio-iodine	P	100	1 °C to 5 °C	2 days	
Radium by other methods	P	100	1 °C to 5 °C	2 months	
Radio-strontium	P	100	1 °C to 5 °C	1 month	
Radio-caesium	P	200	1 °C to 5 °C	2 days	
Uranium	P	50	1 °C to 5 °C	1 month	
Plutonium	P	50	1 °C to 5 °C	1 month	

[a] P = Plastics, e.g. PE (polyethylene), PTFE (polytetrafluoroethylene), PVC [poly(vinyl chloride)], PET [poly(ethylene terephthalate)].

[b] Minimum field sample size for the determination of the specific analyte, based on wet material. In the case where more than one analyte is analysed on the same field sample, a sample mass less than the sum of the masses may be sufficient.

[c] Including duration of transport.

Table 3 — Techniques generally suitable for the preservation of samples for biological and microbiological analysis

Analyte to be studied	Type of container [a]	Minimum sample size [b] g	Preservation and storage conditions	Maximum storage duration [c]	Comments
Benthic macro-invertebrates Macrophytes Algae Phytoplankton Zooplankton Fish	P or G	200	1 °C to 5 °C	24 h	
	P or G	200	Add 3,7 % neutralized formaldehyde (4.7) (see warning)	3 months	Fresh and dry (bio)mass determinations of periphyton and phytoplankton are usually based on the cell volume measurements made during the counting and identification procedure from the preserved sample.
	P or G	200	Add 96 % ethanol (4.5) to give a concentration of 70 % to 75 % (volume fraction)	3 months	
Bacteria, fungi, viruses and parasites	Sterile P or sterile G	100	(5 ± 3) °C, dark and airtight	24 h	
Microbial activity	Sterile G	100	None	24 h	
Toxicity	P or G	1 000	1 °C to 5 °C	24 h	The preservation period will vary according to the method of analysis to be used. See also ISO 5667-16.
	P	1 000	< −18 °C	2 weeks	
WARNING — Beware of formaldehyde vapours. Do not store large numbers of samples in small work areas.					

[a] P = Plastics, e.g. PE (polyethylene), PTFE (polytetrafluoroethylene), PVC [poly(vinyl chloride)], PET [poly(ethylene terephthalate)].
G = Glass.

[b] Minimum field sample size for the determination of the specific analyte, based on wet material. In the case where more than one analyte is analysed on the same field sample, a sample mass less than the sum of the masses may be sufficient.

[c] Including duration of transport.

Annex A
(informative)

Container preparation

A.1 Reagents

A.1.1 Acetone.

A.1.2 Hydrochloric acid, 4 % and 25 % (volume fraction), HCl.

A.1.3 Nitric acid, 10 % (volume fraction), HNO_3.

A.2 Solvent-washed glass containers

WARNING — Organic solvents can be hazardous. Provide suitable handling facilities and handle with care.

Non-disposable sample containers and lids for semi-volatile analysis should be washed with a phosphate-free detergent solution, followed by thorough rinses with hot tap-water and analyte-free water. The last step should be an acetone rinse. The lids should be in place on the container during the rinse step (solvent in the container with the lid tightly screwed down) because the solvents can rinse the plastic from the interior screw threads onto the PTFE lining.

For analysis of volatile organic compounds, sample containers, screw caps, and septa (silicone vapour barriers) should be washed with a phosphate-free detergent, rinsed once with tap-water, rinsed at least twice with analyte-free water, then dried at a temperature greater than 105 °C. A solvent rinse should generally be avoided because it can interfere with the analysis, although a methanol rinse is acceptable.

Alternatively, single use disposable containers and lids may be used for both sample types.

A.3 Acid-washed containers

For trace metal analysis, new sample containers should always be used. Sample containers and lids should be thoroughly cleaned with a phosphate-free detergent solution, thoroughly rinsed with metal-free water, soaked for 24 h in approximately 10 % HNO_3 or approximately 25 % HCl, and rinsed with metal-free water. The acids used should be of at least reagent-grade purity.

A.4 Containers for microbiological samples

Sample containers for the collection of microbiological parameters should be washed with a phosphate-free detergent solution, followed by a thorough rinse with deionized or distilled water, and sterilization by autoclave at (121 ± 3) °C for at least 15 min or by another technique described in ISO 19458. Containers should not produce or release at this temperature any chemicals that would influence biological activity.

Samples that are to be analysed for microbiological analytes may require that containers be sterilized after cleaning. Sterilized containers should be used if sterilized or disinfected sewage samples are to be collected.

A.5 Containers for biological samples

Sample containers for the collection of toxicological or hydrobiological samples should be washed with a phosphate-free detergent solution, triple-rinsed with hot tap-water and should be finished with a 4 % hydrochloric acid rinse. It is possible to use disposable commercial plastics containers, subject to verification of the absence of interference with the analysis. Manipulation of the samples is often necessary, and the optimal methods depend on the study objectives.

Annex B
(informative)

Long-term storage of wet sediment samples using nitrogen vapour freezers

B.1 General considerations

The (long-term) cryogenic storage of specimens using nitrogen vapour freezers (NVF) is a common technology for the conservation of biological and medical tissues. The NVF (a special kind of Dewar) is filled with liquid nitrogen in the bottom. Samples are stored in the gas phase (below −150 °C) on a platform above the liquid nitrogen. It is a cost-efficient and blackout-safe alternative for cryogenic storage compared to ultra-cold electric freezers. A main advantage is the oxygen-free storage to prevent any change or degradation of the samples by oxidation, because the nitrogen replaces air-oxygen. Long-term storage and preservation of environmental samples using NVFs is applied by several environmental specimen bank (ESB) programmes all over the world (e.g. German Environmental Specimen Bank; U.S. Marine Specimen Bank and National Biomonitoring Specimen Bank; Danish Environmental Specimen Bank; Japanese Environmental Specimen Bank) [13] [14]. ESB programmes are storing freshwater and marine aquatic and terrestrial specimens, i.e. soils, sediments, conifer shoots, tree leaves, seaweed, and animal tissues like eggs, fat, kidneys, livers or meat from birds, fishes, mussels or mammals. PTFE-capped glass jars, PTFE jars or stainless-steel containers are commonly used for storing the samples. The primary function of the ESBs is to provide samples for retrospective analyses, i.e. to monitor and control the effects of pollution reduction/restriction programmes like the Stockholm Convention or to recognize emerging pollutants of concern [13] [14] [29].

B.2 Sample collection and handling

Freeze-corers or piston corers equipped with liners are applied for sampling of muddy to sandy riverine and lake sediments within the framework of the German Environmental Specimen Bank [27]. The frozen cores of sampling by means of freeze-corers (up to 1,2 m in length) are washed with demineralized water to remove unfrozen sediment as soon as they are collected. Longer cores are cut to length using a saw with a stainless-steel blade or an angle grinder with a diamond blade. To prevent contamination by abrasion of the blade, the surfaces are washed using demineralized water. The cores are stored as a whole in cylindrical stainless-steel containers (internal diameter: 0,25 m; height: 0,25 m to 0,45 m) in a transportable NVF. Piston core liners are closed with PTFE caps and washed with demineralized water. After that, they are shock-frozen in the NVF using a rack to prevent tilting.

B.3 Sample transport and storage

The samples are transported to the storehouse using a transportable NVF. The storehouse is equipped with NVFs that have a volume of up to 4 m^3 each, for cryogenic storage of environmental specimens. HEPA-filtered clean air conditions in freezer rooms and clean rooms with laminar flow working benches ensure sample stability or contamination during sample preparation (i.e. sub-sampling) and storage. The freezer and room conditions are controlled by computerized security and monitoring systems (i.e. automated level control and refilling of liquid nitrogen to the NVFs). The sediments are stored as a whole or sub-sampled using a diamond blade grinder. The sub-samples are stored in stainless steel containers.

B.4 Long-term stability of samples

The long-term stability of (environmental) samples under deep freezing conditions is validated for a minimum of 17 years of storage [27]. Portions of liver tissue specimens, originally stored in the U.S. National Biomonitoring Specimen Bank, analysed between 1980 and 1987 were re-analysed in 1997 for 17 trace elements. The results indicated that the NVF storage conditions had no noticeable effect on the trace element composition of these samples. Further, the authors expected a decrease in sample moisture because of the low relative humidity in the NVF chambers, but it was not observed.

Portions of spruce shoot samples from two locations, stored in the German Environmental Specimen Bank as wet samples, analysed between 1985 and 1999 using GC-FID (gas chromatography – flame ionization detection) were re-analysed using GC-MS (gas chromatography – mass spectrometry) in 2005 for 16 polycyclic aromatic compounds. The results have shown that there is no significant difference between the original and the re-analysed values (see Table B.1).

Table B.1 — Comparison and testing of original and re-analysed values using the Kolmogorov-Smirnov test

Location	Detection	Number of analysed samples	Minimum	Maximum	Mean	Standard deviation	p-Value
1	GC-FID	72	0,26	111,60	18,64	27,21	
	GC-MS	72	0,27	136,50	18,45	29,90	0,27
2	GC-FID	45	0,12	10,31	2,95	2,96	
	GC-MS	45	0,21	11,02	2,91	3,07	1,0
($\alpha = 0{,}01$; two-sided) (values in µg/kg wet weight)							

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ICS 13.060.30; 13.060.45

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