SLOVENSKI STANDARD
oSIST prEN 16698:2014

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Kakovost vode - Navodilo za kvantitativno in kvalitativno vzorčenje fitoplanktona v celinskih vodah

Water quality - Guidance on quantitative and qualitative sampling of phytoplankton from inland waters

Wasserbeschaffenheit - Anleitung für die quantitative und qualitative Probenahme von Phytoplankton aus Binnengewässern

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13.060.10	Voda iz naravnih virov	Water of natural resources
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Water quality - Guidance on quantitative and qualitative sampling of phytoplankton from inland waters

Qualité de l'eau - Lignes directrices sur l'échantillonnage quantitatif et qualitatif du phytoplancton dans les eaux intérieures

Wasserbeschaffenheit - Anleitung für die quantitative und qualitative Probenahme von Phytoplankton aus Binnengewässern

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Contents

Foreword

This document (prEN 16698:2013) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of the Water Framework Directive (2000/60/EC) (WFD), and the Directive on Environmental Quality Standards (Directive 2008/105/EC).

WARNING —Working in or around water is inherently dangerous, Persons using this European standard should be familiar with usual field and 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 health and safety practices and to ensure compliance with any national regulatory guidelines.

IMPORTANT — It is absolutely essential that tests conducted according to this European Standard be carried out by suitably trained staff.

Introduction

Phytoplankton samples provide information on the taxonomic composition as well as the spatial density of the individual taxa and their relative abundances. They allow the calculation of the productivity and biomass of the phytoplankton assemblage as a whole as well as for the individual taxa.

For the purpose of limnological investigations and monitoring of surface waters representative phytoplankton samples are necessary. Therefore suitable sampling methods are needed depending on the aims of the investigation and the given natural conditions.

1 Scope

This European standard gives guidance for sampling of phytoplankton from inland waters for quantitative and qualitative limnological investigations and monitoring of water quality, e. g. ecological status.

This European standard is intended to ensure uniform conditions for phytoplankton sampling in inland waters and describes methods of sampling techniques for phytoplankton in inland waters (rivers, channels, lakes, ponds, reservoirs and other artificial water bodies).

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15204, *Water quality – Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique).*

EN 14996, *Guidance on assuring the quality of biological and ecological assessments in the aquatic environment.*

EN ISO 7027, *Water quality – Determination of turbidity (ISO 7027).*

EN ISO 5814, *Water quality – Determination of dissolved oxygen; electrochemical probe method (ISO 5814).*

3 Terms and definitions

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

3.1
holomictic lake
lake that shows complete mixing of the water body every year

3.2
monomictic lake
lake with uniform water temperature and holomictic conditions during the autumn to spring phase (no ice cover in winter) during which the water body is completely circulated

3.3
dimictic lake
lake with uniform water temperature and holomictic conditions twice a year: during autumn (before ice cover) and during spring (after ice cover)

3.4
polymictic lake
shallow lake which has more than two circulation phases per year comprising the whole water body

3.5
shallow lake
polymictic lake with a maximum depth usually ≤ 10 m

3.6
mixing of water
complete mixing of the whole water body

Note 1 to entry: Complete mixing of water is only possible when the density in the whole water column is equal, that means usually the temperature is the same in every depth.

Note 2 to entry: In the context of this European Standard shallow lakes are defined as polymictic lakes.

3.7
metalimnion
thermocline
water zone with the greatest vertical density gradient caused by temperature gradients in a lake > 1 °C/m

3.8
stratification
state of a water body during which a vertical density gradient in the water column prevents its complete mixing

3.9
epilimnion
z_{epi}
zone of the water body between surface and thermocline in which the water temperature and density is approximately uniform, showing a temperature gradient of < 1 °C/m

3.10
euphotic zone
z_{eu}
zone of the water body below the surface, in which the photosynthetic production takes place

Note 1 to entry: Layer with more than 1% of incident light intensity (or subsurface light see [11]). The euphotic depth is approximately 2,5fold Secchi depth [1], with exception of humic lakes or lakes showing algal scums (0,8 to 3,0 fold Secchi depth) [11]

3.11
mean depth
depth value (in metres) obtained from dividing a lake's volume (m^3) by its surface area (m^2)

3.12
integral sampler
water sampler which provides a representative sample of a defined continuous water column

3.13
Secchi depth
maximum depth at which a white disk according to Secchi that is lowered into the water is still just visible; it is the average of the depth at which the disk being lowered disappears and the depth at which it reappears when it is subsequently raised

3.14
stream centre line
connecting line between the positions of the main flow in transsects of a running water

4 Principles of phytoplankton sampling

4.1 General

A representative phytoplankton sample is taken and preserved for later microscopic investigation (e.g. according to EN 15204). Generally, it is distinguished between sampling in lakes and sampling in rivers. In rivers that are completely mixed, it is sufficient to take a sample from the water column in the stream centre line.

In temporarily stratified waters the mixed water layer (epilimnion) or the zone in which phytoplankton is produced (euphotic zone) shall be sampled. In these waters, a vertical mixed sample shall be taken. This is possible either with an integral sampler or by mixing of sub-samples from all depths of the mixed or euphotic water column.

In clear lakes a mixed sample shall be taken from the euphotic zone. In turbid and humic lakes it is adequate to take the sample from the mixed zone (epilimnion in stratified lakes, whole water column in polymictic lakes). The depth of these zones shall be determined by recording Secchi depth, and depth profiles of water temperature and dissolved oxygen. The distribution of the phytoplankton should be determined by measuring chlorophyll-a using a fluorescence sensor.

For special objectives samples may be collected individually from fixed depths (e.g. drinking water intake).

4.2 Selection of sampling sites

4.2.1 Sampling sites in rivers and streams

The width of the river at the sampling point may not be larger than twice the mean width in adjacent unobstructed sections (i. e., sections without bank fixation). The average cross-sectional depth may not exceed the depth of the unobstructed upstream section by more than one third. Sampling shall take place in the stream centre line. Samples from large and deep rivers may be taken from a bridge or a boat.

Complete mixing of the water column shall be confirmed by vertical measurements of temperature and chlorophyll fluorescence. Artificially widened and consolidated stream sections (harbours, locks, sites directly before or behind barrages etc.) are no representative sampling points for phytoplankton in rivers since the flow velocity at these places is highly modified, which may result in stratification or sedimentation of the phytoplankton. River sections that are not completely mixed, like harbours and impoundments, shall be sampled according to the instructions for lakes (see 4.2.2 and 6.3).

4.2.2 Sampling sites in lakes

In lakes the sampling of phytoplankton shall take place at the bathymetric deepest point (z_{max}) because the depth profiles of temperature and oxygen shall as well be taken at the point of maximum depth. Sampling at a single station at the centre of the lake may be sufficient [2], [13], but may differ from the deepest point. In lakes that are segmented by a distinctive ground relief forming several basins, sampling generally shall be performed in each basin. The samples from each basin shall be treated separately, i. e. they shall not be pooled with samples from another basin. Lakes with longitudinal gradients (reservoirs, channel lakes) shall be sampled at different points reflecting the biological differences to be expected between the sampling points (e. g., in reservoirs: riverine zone, transitional zone and lacustrine zone [4]).

Only in exceptional cases (e. g. when no boat can be hoisted because of very steep shores) sampling can be conducted from the shore line or outflow respectively. These samples are not vertically integrated and are restricted to qualitative or semiquantitative results with regard to the phytoplankton community of the surface water body. No additional information on stratification, light climate and metalimnic or hypolimnic nutrients and deep chlorophyll maxima (DCM) are possible.

4.2.3 Horizontal patchiness in lakes

It is well known that in lakes patchiness (small-scale spatial variability of the phytoplankton abundance and/or species composition) can occur. For some monitoring purposes it is advisable to integrate samples over some area, thus removing the variability within the area integrated. The loss of information caused by this pooling of samples should be weigthed against the gain in accuracy achieved by the reduction of an error component [3]. If the objective is to investigate the patchiness, all samples shall be taken using the same sampling method.

In valley lakes, especially in reservoirs, the phytoplankton biomass is lower at a point near the outflow or dam than in the longitudinal centre of the lake. In this case sampling at the end of the lake would lead to an underestimation of the phytoplankton biomass and trophic level of the lake [4]. Therefore, in valley lakes at least one additional point at the longitudinal centre shall be sampled. In large lakes (> 100 ha or 1 km^2) more than one sampling point is recommended to take into account differences in the phytoplankton composition that may occur along the longitudinal axis of the water body. Strongly isolated bays shall also be sampled separately [2].

4.3 Sampling frequency and replicates

The sampling frequency is very important. The required number of samples per year depends on the objectives of the investigation, the intended type of data evaluation and the duration of the period without ice cover. During the growing season (March/April to October) at least monthly sampling is recommended. For a reliable assessment of a water body's ecological quality, an investigation period of at least three years is advisable. If necessary, the sampling frequency may be reduced in accordance with the details given in Table A.2.

Depending on the objectives of the investigation and taking into account the seasonal processes in the water body (spring peak, clear water phase, stratification period, full mixing etc.) the maxima of phytoplankton development shall be covered. The probability of trophic mis-classification depends on the frequency of sampling (see Annex A and [12]).

Replicates are required when it is an objective of the investigation to get information on the variability of phytoplankton biomass (or chlorophyll content) results received from the individual samples taken from one sampling point. In regular monitoring programs replicates are not necessary, but it is recommended to take several samples from one sampling point and pool them.

5 Equipment and preservatives

5.1 Equipment for sampling

To prevent spreading of flora and fauna between water bodies, the sampling equipment shall be cleaned between samplings in different water bodies. After each day of sampling, plankton nets shall be washed in warm freshwater with detergent or in an ultrasonic water-bath in order to reduce clogging and ensure optimum filtration capacity. Equipment such as water samplers, hoses, plankton nets and mixing containers shall be rinsed in freshwater and dried before they are stored for future use. Hoses shall be hung up to dry. Rapid drying is essential to prevent unwanted growth within hoses.

5.1.1 Suitable water sampler. See Annex A for examples of suitable samplers.

5.1.2 Electrochemical probe with depth sensor to measure water temperature and dissolved oxygen according to EN ISO 5814.

5.1.3 Secchi disk

5.1.4 Chlorophyll fluorescent probe with depth sensor (optional).

5.1.5 Boat, suitable for local conditions with appropriate safety equipment.

Equipment for communication from the boat to designated shore-based staff, with access to rescue services is recommended. An experienced boat driver familiar with local conditions should be deployed.

5.1.6 Detailed bathymetric maps of the surveyed area

5.1.7 Echo sounder, to localize the deepest point of a lake

5.1.8 Winch, with counter or rope with metre marks for deep lakes.

5.1.9 Mixing container, for example a plastic can or bucket with lid, for homogenising integrated samples.

5.1.10 Plankton net, mesh width 10 µm to 25 µm.

5.1.11 Sample bottles, with a volume of 100 ml, 250 ml or 500 ml.

Use clear transparent narrow-neck glass bottles with a volume of 100 ml, 250 ml or 500 ml (see 6.6).

5.1.12 Dark closed containers, suitable for the transport of the sample bottles (e.g. cooling boxes).

5.2 Fixatives and preservatives

Two different reagents shall be used for phytoplankton sample preservation (for details see EN 15204).

5.2.1 Alkaline Lugol's iodine, with sodium acetate for preservation of plankton from neutral or alkaline waters (pH ≥ 7).

5.2.1 Acidic Lugol's iodine, with acetic acid for preservation of plankton of acidic waters (pH < 7).

5.2.3 Ethanol, C_2H_5OH, volume fraction 90% to 96 %, for preservation of diatom samples.

6 Procedure

6.1 General requirements for phytoplankton sampling

A representative phytoplankton sample shall be taken using a suitable water sampler (see Annex A for examples). During sampling, the sampling gear shall not be allowed to touch the bottom, because this might contaminate the water samples.

The phytoplankton sample shall be filled immediately or after mixing in sampling bottles and preserved for later microscopical investigation according to EN 15204.

Sample bottles shall be marked before sampling to avoid mixing up the samples. Waterproof marker pen or pencil shall be used (ballpoint pens or regular marker pens are not appropriate). Writing directly on the glass is not recommended. Instead, the use of water-resistant adhesive tape that can be labelled is advisable.

As a minimum, the following information shall be given for each sample (included on the label or in the sampling protocol):

- name of locality;
- sampling person,
- order number or order designation;
- sampling date and time;
- sampling position if appropriate;
- sampling depth (mixed, epilimnetic or euphotic depth);
- type of sample (e. g. phytoplankton or pelagic diatoms; in case of net samples mesh width and volume of water filtered through the net).

The sample shall be labelled to ensure that it can be clearly identified using the information given in the sampling protocol.

The sample bottles shall be transported to the laboratory in dark, and if necessary cooled, non-transparent containers.

The sub-samples for phytoplankton and other parameters (e. g. chlorophyll-a and nutrients) shall be taken from the same mixed sample. This means that the sample volume should be sufficiently large. Nutrient analyses shall only be done from mixed euphotic samples, if the euphotic zone does not reach into the hypolimnion. If the euphotic zone stretches into the hypolimnion a separate mixed sample from the epilimnion shall be taken for nutrient analysis.