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Kakovost vode - Navodilo za kvantitativne in kvalitativne preiskave morskega fitoplanktona

Water quality - Guidance on quantitative and qualitative investigations of marine phytoplankton

Wasserbeschaffenheit - Anleitung für die quantitative und qualitative Untersuchung von marinem Phytoplankton

Qualité de l'eau - Guide pour l'étude quantitative et qualitative du phytoplancton marin

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Water quality - Guidance on quantitative and qualitative investigations of marine phytoplankton

Qualité de l'eau - Guide pour l'étude quantitative et qualitative du phytoplancton marin

Wasserbeschaffenheit - Anleitung für die quantitative und qualitative Untersuchung von marinem Phytoplankton

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Foreword

This document (EN 15972:2011) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 2012, and conflicting national standards shall be withdrawn at the latest by March 2012.

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

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

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Introduction

Investigations of phytoplankton are an important part of marine environment monitoring. Phytoplankton responds rapidly to environmental changes (supply of nutrient salts, climate, light access etc.), and monitoring of occurrence, species composition and biomass may therefore in many cases be used to characterise the environment and the degree of impacts. The European Union Water Framework Directive therefore requires monitoring of marine phytoplankton as a part of assessments of ecological conditions. Investigations of phytoplankton are also included in monitoring programmes connected with other European directives (Urban Waste Water Treatment Directive, Habitats Directive), international conventions and national regulations. Monitoring of harmful/toxic phytoplankton is carried out both for the aquaculture industry and in connection with authorities' control of organisms for human consumption (shellfish). This requires uniform procedures for collection and quantification of phytoplankton.

Most principles for characterisation of environmental conditions based on phytoplankton require the use of quantitative methods, i.e. that the occurrence of species and quantities can be related to a known water mass. Data interpretation further requires information on the physical and chemical properties of the water body (supporting parameters). In order for environmental authorities to utilise the information, it is important that the investigations are comparable in space and time and that the data is of high scientific quality. This European Standard focuses on a limited selection of methods that can be documented precisely, reproduced and which have been in use for some time. This European Standard does not comprise chlorophyll determination. For chlorophyll determination see ISO 10260 [1].

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

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1 Scope

This European Standard gives guidance for sampling, preservation, storage, quantification and qualitative analysis of phytoplankton from marine waters. Guidance for quantification is limited to the use of light microscopy with phase-contrast and epifluorescence.

This European Standard specifies:

- the development of the sampling programme;
- requirements for sampling equipment;
- procedures for sampling and treatment of samples in the field;
- methods for quantification;
- qualitative analysis.

This European Standard describes minimum requirements for environmental monitoring.

EN 15972:2011 (E)**2 Normative references**

The following referenced documents are necessary for the application of this document. For dated references, only the cited edition 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)*

3 Terms and definitions

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

3.1 euphotic zone
upper zone of a water body which is penetrated by light, and where the primary production over one or more days is equal to or greater than the respiration, i.e. a positive net production

NOTE Empirically, the euphotic zone is considered to extend down to the depth where 1 % of the surface light is present.

3.2 phytoplankton
community of free-living, suspended, mainly photosynthetic organisms in aquatic systems comprising Cyanobacteria and microscopic algae

[EN 15204:2006, 3.10]

3.3 phytoplankton biomass
mass of living matter comprising phytoplankton

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NOTE The phytoplankton biomass can be expressed in terms of chlorophyll content, the carbon content of the algae (algal carbon) or the volume of the phytoplankton (biovolume).

3.4 qualitative sample
phytoplankton sample collected by plankton net, i.e. a concentrated sample where the size of the phytoplankton is mainly larger than the mesh diameter

NOTE Allows for recordings of taxon/taxa occurring in low concentrations.

3.5 quantitative sample
phytoplankton sample where a part of a body of water is enclosed so that the density of each taxon and their relative abundance per unit volume may be recorded

3.6 receiving water body
water body that receives an input of material, of either natural or anthropogenic origin

NOTE The term often appears in the context of anthropogenic input, for example, effluent from municipal waste water outlets or industrial processed water.

[EN ISO 16665:2005, 2.4]

3.7**sampling station**

precise location where samples are collected

NOTE A sampling station is defined by its geographical position (OS National Grid Reference, latitude, longitude), its depth (relative to chart data and normalised to mean low water as given in tide tables) and any other invariant of physical conditions. The station is delineated using the given level of precision. In cases of doubt when revisiting sampling stations, emphasis should be placed on landmarks and water depth.

[EN ISO 16665:2005, 2.7]

3.8**stratification**

two or more water bodies with different physical properties (temperature, salinity, density) lying upon each other

NOTE A thermocline separates the water bodies from each other.

3.9**thermocline**

boundary layer between two horizontally arranged water bodies with different physical properties which change dramatically in the vertical

3.10**water body**

body of water, standing or flowing; a lake, river, or other collection of water

3.11**water transparency**

estimate of turbidity determined by recording the depth at which a white disc with black and white quarters lowered into the water is no longer visible from the surface¹²

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4 Aim and strategy for sampling phytoplankton**4.1 Sampling programme**

Before a survey is carried out, the sampling programme shall be defined according to the individual aims of the survey and the required precision of results and their intended use. Sampling methodology, number of sampling stations, sampling frequency and methodology for sample processing will vary between different types of investigations. During development of the programme, consideration shall be given to hydrographic conditions in the survey area, information about local pollution sources, knowledge from previous surveys and any other conditions that may be of importance for the phytoplankton composition in the survey area.

For long-term baseline studies, it should be ensured that methodological changes do not have any influence on the results of time series.

4.2 Types of surveys**4.2.1 Pilot survey**

Pilot surveys shall provide a general overview of the occurrence of phytoplankton and physical and chemical conditions in the water body of the survey area. In areas where larger scale environmental surveys or monitoring is required, there can be a need to carry out a pilot survey in advance. This pilot survey shall provide the necessary information for developing more specific sampling programmes. The requirements for equipment, methodology and repeatability are usually relatively simple. The phytoplankton samples shall be collected by plankton net and/or water samplers. Hydrographic data shall be collected with a continuous

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recording probe or by use of a water collector and subsequent analysis of sampled water. The phytoplankton samples are processed in such a way that the most important taxon/taxa can be identified and quantified.

NOTE In this context, the most important taxon/taxa are those that are dominant in terms of numbers and/or biomass and taxon/taxa that have characteristic properties (indicators of eutrophication, producers of toxins etc.).

4.2.2 Baseline survey and monitoring of biological diversity

Baseline surveys should be carried out for environmental monitoring and characterisation of biodiversity. Detailed analyses should be carried out on the relative species composition and abundance within the phytoplankton communities. The investigation shall provide the basis for characterising environmental conditions in the relevant areas in accordance with set criteria or by comparison with phytoplankton communities in other representative areas. The requirements for scientific documentation and ability to be replicated are usually relatively high.

If the baseline survey is carried out over time in one area, the investigation will provide a temporal description of natural variations of the phytoplankton communities and document any gradual changes (trend monitoring). The investigation shall be carried out at fixed stations and follow a carefully defined programme. At the start of investigations, in areas where the hydrographic conditions are insufficiently known, a preliminary investigation of the hydrographic conditions shall be carried out to identify the different water masses within the water column and to locate the thermocline depth before the formal sampling programme is established.

The investigations shall be carried out using quantitative methods. There should be specified requirements for station positioning, sampling depth, frequency and supporting parameters, such as chlorophyll-a concentration hydrographic measurements, nutrient analyses, *in situ* fluorescence and water transparency. Methods should be chosen so that the data can be used as a basis for comparison with baseline surveys in other areas. Additional qualitative samples (plankton net samples) can be taken to give a complete description of the species composition.

In areas where potential changes in environmental conditions are to be monitored, such as in impacted receiving waters or areas where there are plans for establishing activities that may cause environmental impacts, an initial baseline survey shall be carried out, and thereafter follow-up monitoring surveys.

4.2.3 Specialised surveys - Monitoring of selected taxon/taxa

Specialised surveys are recommended for monitoring toxic and harmful algae (phytoplankton) for fish in aquaculture and shellfish production (such as blue mussels). Sampling shall be carried out at fixed locations and according to defined programmes where sampling depth, frequency and methodology are described. During sample processing, the occurrences of previously specified taxon/taxa shall be quantified. The investigations shall provide the basis for issuing warnings or to set in motion strategies for hazard reduction (mitigation).

4.2.4 Verification survey

Verification surveys may be used to verify phytoplankton blooms detected by remote sensing techniques, for example in cases of discoloured water or on suspicion of mass occurrences of harmful algae (phytoplankton). The samples are collected by using a fine meshed plankton net and/or water samplers and further processed to identify the dominant taxon/taxa.

4.3 Sampling stations

Sampling stations shall be representative for the areas to be investigated or monitored. Back-eddies, more or less enclosed bays or other areas that are topographically or hydrographically anomalous shall be avoided unless the aim is specifically to investigate these.

If the area has been investigated previously or there is ongoing biological, physical and/or chemical sampling, the same stations shall be used, unless those stations will not be able to serve the current purposes.

Sampling should preferably be carried out from a boat. If sampling from quay or pier is necessary, there shall be sufficient water depth to avoid influence from the bottom sediment.

When using this means of sampling, the samples can become contaminated by micro-algae associated with hard substrates such as fouling on the quay/pier, macroalgae etc. The samples shall therefore be taken upstream and as far from the edge of the quay/pier as possible. For baseline surveys in receiving waters from industry, municipal discharges and aquaculture activities, the samples shall, as far as possible, be taken upstream of the plant(s). Sampling at a single station is usually sufficient. However, in particular or acute situations, sampling shall be carried out according to continual assessment of the most beneficial station positioning.

4.4 Sampling depth

In the case of a monitoring network operated in an international framework, sampling strategy shall be elaborated by the expert group of the member states. Samples for quantitative analysis may be collected either individually from fixed depths, as combined samples where samples from different depths are mixed together to form a single sample or as integrated samples, where sampling encompasses the entire water column.

Combined samples may be used in cases when an overview of the numerical dominating species is required and where it is not necessary to link the occurrence of phytoplankton to hydrographical conditions or chemical parameters.

In marine areas, where large fluctuations in the physical parameters within the water column occur, such as fjords or brackish waters, the samples should be taken both above and below the thermocline, because they can often form a boundary between different phytoplankton stocks. In marine areas where different water bodies meet, hydrographic measurements shall be carried out to identify stratified water layers of various origins down the water column, in order to ensure that the different water masses are adequately sampled.

For baseline surveys or trend monitoring where the phytoplankton occurrences are related to chemical parameters such as nutrient levels, samples usually shall be taken from fixed depths. Standard sampling depths within the upper 100 m of open sea (recommended by ICES¹) are 0 m, 5 m, 10 m, 20 m, 30 m, 50 m, 75 m and 100 m. This should be the case in areas where no vertical stratification is found. In fjord areas, additional sampling shall be carried out at 2 m. The number of sampling depths may be increased if there is information from, for example, hydrographic measurements on increased frequency of phytoplankton occurrence other than the recommended depth. If *in situ* fluorescence is measured, an additional sample may be collected from the depth at which the fluorescence maximum occurs. See A.2.2 for a description of fluorescence maximum.

The sampling depth from which samples are to be processed should be decided on an individual basis, based on the aim of the investigation. As a minimum, at least one water sample from every sampling date shall be processed. Samples from the same depths shall be processed throughout the entire duration of the programme, to ensure comparable data. Additional samples can be processed if necessary to get information about the phytoplankton composition at the thermocline or other interesting levels in the water column.

Integrated samples are often used in connection with specialised surveys and monitoring of particular taxon/taxa. For example, for monitoring of toxic or harmful phytoplankton in connection with fish or shellfish production, integrated samples shall be taken within the depth intervals at which the fish or shellfish are located if vertical stratification occur in the height of water concerned by the aquaculture livestock.

The net samples are used to complete the total species spectrum and for the determination of difficult taxa. The precise depth, or depth interval, at which the haul shall be carried out, shall be decided based on the aims of the sampling programme and/or the hydrographic conditions at the sampling location.

¹ International Council for the Exploration of the Sea

EN 15972:2011 (E)**4.5 Sampling frequency and duration**

For baseline surveys monitoring, sampling shall be carried out at least twice per month. Sampling may be carried out more frequently during periods of the year where significant variations occur over short time-periods, for example during spring. The investigation shall cover the growth season of the phytoplankton over a period of one year. In many areas, the growth season actually covers the whole year.

For trend monitoring and studies of biodiversity, the sampling frequency shall be as for baseline surveys, but in addition, monthly sampling shall be carried out during the winter period. Investigations are recommended to be carried out over a period of at least 6 years, to document annual variations. However, this frequency may be determined by statistical analysis of the data and the needs for the survey.

In the case of specialised surveys for monitoring toxic phytoplankton in connection with health warnings about shellfish consumption, the sampling frequency shall comply with the current regulations set by the relevant authorities. For other specialised surveys, the sampling frequency and sampling period shall be adjusted according to the aims of the investigation.

4.6 Parallel water samples

In many cases, it may be appropriate to go back to previously collected samples to verify identifications and/or quantifications, split previously grouped taxa to lower taxonomic levels or to carry out other analyses of the collected material. Degradation of biological material will begin if sample containers are opened and air is introduced. Therefore, parallel samples preserved with buffered formaldehyde taken for future reference purposes shall be kept in separate containers. When planning the sampling programme it should be decided whether parallel water samples are to be collected.

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5 Equipment**5.1 Equipment for quantitative sampling**

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Quantitative samples determining the density of the various taxon/taxa shall be collected using a water sampler, pipe or hose.

All water samplers shall be constructed so that they can be filled with water from a specified depth/depth interval. All water samplers should be made of non-toxic material. When using a hose, the lower end shall be sufficiently weighted to ensure that the hose is positioned vertically in the water column, and correctly takes an integrated sample from the relevant part of the water column. For an overview of various types of water samplers, see Annex B.

Additional equipment:

- weight for triggering closure of the water sampler;
- winch with counter or rope with metre marks;
- shackle to attach the wire/rope to the water sampler;
- mixing container, e.g. a plastic can or bucket with a lid for homogenising integrated samples.

5.2 Equipment for qualitative sampling

Qualitative samples for investigations of species occurrences should be taken using a plankton net, see Annex C.

In baseline surveys, where occurrences of small phytoplankton are important, a plankton net with mesh size from 5 µm to 10 µm shall be used to ensure that both nanoplankton and microplankton are sampled. For