SLOVENSKI STANDARD
SIST EN 13946:2003

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Kakovost vode - Navodilo za rutinsko vzorčenje in predpripravo rečnih bentoških kremenastih alg

Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers

Wasserbeschaffenheit - Leitfaden zur Probenahme und Probenaufbereitung von benthischen Kieselalgen in Fließgewässern

Qualité de l'eau - Guide pour l'échantillonnage en routine et le prétraitement des diatomées benthiques de rivieres

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13.060.10	Voda iz naravnih virov	Water of natural resources
13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water

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EUROPEAN STANDARD

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English version

Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers

Qualité de l'eau - Guide pour l'échantillonnage en routine et le prétraitement des diatomées benthiques de rivières

Wasserbeschaffenheit - Leitfaden zur Probenahme und Probenaufbereitung von benthischen Kieselalgen in Fließgewässern

This European Standard was approved by CEN on 21 February 2003.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

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

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Contents

Foreword

This document (EN 13946:2003) 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of an identical text or by endorsement, at the latest by November 2003, and conflicting national standards shall be withdrawn at the latest by November 2003.

Annex A is informative.

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

Introduction

WARNING — Persons using this European 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 health and safety practices and to ensure compliance with any national regulatory conditions.

Diatoms are an important component of aquatic ecosystems and constitute a water quality monitoring tool where the primary objective is either a measure of general water quality or of specific components of water quality (e.g. eutrophication, acidification). The requirement for the monitoring of such processes is inherent in the Water Framework Directive (2000/60/EC) and Urban Waste Water Treatment Directive (91/271/EEC) in addition to other EU Directives and international agreements. This European Standard covers aspects of sampling and pre-treatment relevant to assessment of water quality using benthic diatoms. Some aspects may also be relevant to measures of ecological integrity. These sampling instructions will result in samples suitable for quantifying relative numbers of benthic diatom taxa present. If it is necessary to quantify absolute numbers of taxa, or fresh weight per unit area, modifications to the method are required, which are not within the scope of this standard.

The use of diatoms as indicators of river water quality is widely accepted both in Europe and the USA. The methodology is based on the fact that all diatom species have tolerance limits and optima with respect to their preference for environmental conditions such as nutrients, organic pollution and acidity. Polluted waters will tend to support an increased abundance of those species whose optima correspond with the levels of the pollutant in question. Conversely, certain species are intolerant of elevated levels of one or more pollutants, whilst others can occur in a wide range of water qualities.

Methods using diatoms to assess water quality have been developed in several European countries (recent work is summarized in the proceedings of three symposia [1 to 3]. The methodologies for evaluating the diatom data vary but the sampling and pre-treatment processes are similar [4].

According to the precise usage to which this standard is to be put it is essential for specifiers and users to agree on any necessary variations or optional procedural details prior to use.

All numerical values given in this standard are approximate.

1 Scope

This guidance European Standard establishes a method for the sampling and laboratory preparation of benthic diatoms for water quality assessments. Data produced by this method are suitable for production of water quality indices based on the relative abundance of taxa. With appropriate modifications the method can be applied to the study of benthic diatoms in lakes.

2 Principle

Benthic diatoms from submerged hard surfaces or submerged macrophytes in rivers or streams are sampled in order to produce representative collections of the diatom assemblage indicative of water quality. Samples are cleaned using strong oxidizing agents in order to prepare diatoms for identification and enumeration.

The data obtained from the microscopic analysis of these samples are suitable for the production of diatom-based water quality indices (see references 1, 2, and 3).

3 Terms and definitions

For the purposes of this European Standard, the following terms and definitions apply:

3.1
artificial substratum
substratum introduced into river by operator specifically for colonisation by diatoms

3.2
benthic diatoms
diatoms living on substrata, rather than suspended in the water column

3.3
boulder
mineral substratum with a diameter > 256 mm

3.4
cobble
mineral substratum with a diameter > 64 mm and ≤ 256 mm

3.5
euphotic zone
the part of the water column in which there is sufficient light for photosynthesis

3.6
frustule
cell wall of diatoms, composed of silica and consisting of two valves linked by two or more girdle bands

3.7
habitat
the specific environment in which an organism lives

3.8
pebble
mineral substratum with a diameter > 16, ≤ 64 mm

3.9
riffle
shallow part of a stream with swift flow, usually with a broken surface

3.10
substratum
natural or non-natural material from which benthic diatoms are sampled

3.11
taxa
taxonomic units, for example families, genera or species

3.12
valve
structural component of the diatom frustule (3.6)

4 Equipment

4.1 Field sampling

— Appropriate water safety equipment;

— Waders;

— Stiff toothbrush (or other similar instrument) or knife (or other suitable blade);

— Plastic tray (approximately 30 cm × 20 cm or larger);

— Sample bottle with a tightly fitting lid;

— Indelible marker pen (or other means of labelling samples). If labels are used, these shall be capable of surviving wet conditions;

— Hoe, with a fine-meshed net attached and a long handle(if vertical hard surfaces are to be sampled);

— A glass-bottomed box or bucket (“Aquascope”) is useful for finding suitable substrata under some circumstances.

4.2 Laboratory

See annex A.

5 Reagents

5.1 General

Reagents used in the preparation of the diatom frustules need not be of analytical grade but should be of a quality appropriate for the digestion process.

5.2 Preservatives

These are required to stop cell division of diatoms and decomposition of organic matter. No preservative is necessary if the sample is to be processed within a few hours of collection, as long as steps are taken to minimize cell division (i.e. by storage in cool, dark place). Lugol’s iodine can be used for short-term storage; however, it is not suitable for long-term storage, due to problems caused by sublimation. Buffered formaldehyde or ethanol are recommended for long-term storage of samples. Samples can also be deep-frozen.

5.2.1 General

5.2.2 Buffered 4% v/v (minimum) formaldehyde (HCHO) solution

Dilute a stock solution of formaldehyde to 4 % in a solution buffered to pH 7. Suitable buffers include HEPES (*N*-2-hydroxymethylpiperazine-*N*-2’-sulfonic acid), borate and hexamethylenetetramine.

A final solution of 1 % to 4 % (v/v) is recommended (the quantity required will depend upon the amount of organic matter present in the sample).

NOTE The buffer is necessary to prevent dissolution of the silica frustules.

5.2.3 Lugol's iodine

Dissolve 2 g potassium iodide and 1 g iodine crystals in 300 ml distilled or demineralised water. The resultant liquid should be straw coloured. It should be stored in an air-tight and light-proof container to minimise sublimation.

Add 1 to 5 drops of Lugol's iodine per 100 ml sample to give a final "straw" colour. More may be necessary if samples are rich in organic matter.

NOTE Some recipes for Lugol's iodine include acetic acid or glutaraldehyde to prevent loss of flagella. These reagents should be omitted when the solution is to be used for diatoms, as they can lead to the dissolution of silica.

70% Ethanol (C_2H_5OH) can also be used for this purpose.

5.3 Reagents for cleaning diatoms

See annex A.

5.4 Reagents for preparing permanent slides

A diatom mountant with a refractive index > 1,6 is required. Proprietary brands include Naphrax and Hyrax.

6 Procedure

6.1 Choice of substratum

Diatoms can be found growing on most submerged surfaces; however, the composition of the community varies depending upon the substratum chosen. Ideally, a single substratum should be used at all sites included in a survey.

Areas of the river bed with naturally occurring moveable hard surfaces (large pebbles, cobbles and boulders) are recommended wherever possible. If such hard surfaces do not occur naturally, then it is possible to sample vertical faces of man-made structures such as quays and bridge supports (so long as these are not made from wood). Other man-made hard surfaces, such as bricks can also be sampled, if these have been in the river for long enough to ensure that assemblages are in equilibrium with their environment. At least four weeks is recommended but the period depends upon environmental conditions. See also comments in 6.3.3.

In deeper rivers where the underlying substrata are finer silts and sands (and when no hard substrata are available) consideration should be given to the introduction of artificial substrata within the euphotic zone.

Samples of diatoms can also be collected from submerged macrophytes. Where possible, comparative studies in rivers should be based on samples collected from the same macrophyte species (or group of morphologically similar species).

6.2 Sample site selection

A segment of river that has substrata suitable for sampling should be selected. As a general rule, this should be about 10 m in length, but longer lengths may be appropriate, depending upon the physical uniformity of the river and the availability of substrata. "Riffles" are preferred, as these tend to have a good variety of natural hard surfaces (6.1).

A detailed description of the site (location, width, depth, substratum type, percent cover of macrophytes, shade etc.) is required on the first occasion that a sample is collected. A photographic record is also recommended. This information serves as an aid the interpretation of data and to help future samplers locate the site. On subsequent visits, notes may be limited to major changes that have occurred since the previous visit, and any variations in sampling protocol employed.

6.3 Sampling methods

6.3.1 Moveable natural hard surfaces

In general, cobbles are the preferred substratum for sampling, as these balance substratum stability (allowing diatom communities to develop) with manoeuvrability. Pebbles and boulders can also be used. At least five cobbles should be sampled. However, if cobbles are unavailable, then either 5 small boulders or 10 pebbles should be sampled. An area of approximately 10 cm^2 or more should be scraped. If fewer suitable substrata are available, then a note should be made to this effect.

The following microhabitat conditions should be fulfilled:

1) areas of heavy shade should be avoided (if it cannot be avoided, then a note should be made to this effect). Areas very close to the bank should also be avoided;

2) the substrata shall be submerged for long enough to ensure that assemblages are in equilibrium with their environment. At least four weeks is recommended but the period depends upon environmental conditions. The precise depth is unimportant so long as the surfaces have not been exposed to air. All depths that can be easily sampled wearing waders are usually suitable, so long as these remain in the euphotic zone;

3) in general, samples should be collected from within the main flow of the river at the sample site. Zones of very slow current (approx. ≤ 20 cm s^{-1}) should be avoided as these allow the build-up of loosely attached diatoms, silt and other debris.

Collect a selection of substrata from a variety of locations within the sample site, which fulfil the microhabitat requirements listed above. Where suitable substrata are very abundant, random or stratified sampling strategies may be appropriate within a defined sample site.

Remove any loosely attached surface contamination (e.g. organic debris) by washing the substratum briefly in the stream water. Place the substrata in a tray, along with approximately 50 ml of river water.

Wash a stiff toothbrush in clean river water and rub it on a clean surface in order to minimise any diatom contamination from previous samples. Brush the upper surface of the substratum vigorously to remove the diatom film, rinsing the toothbrush periodically in the water in order to transfer the diatoms.

A knife or other sharp instrument can also be used to remove the diatom film. This will be more effective at removing firmly-attached diatoms, but will be less efficient at penetrating crevices on rough surfaces, may cause more damage to frustules and may lead to more rock particles being transferred to the sample. However, it is unlikely that there will be any quantitative difference in results. The knife should also be rinsed in river water and cleaned before use.

Alternatively, the diatom film can be removed using a toothbrush or knife and washed directly from the surface of the substratum into a sample bottle. The toothbrush or knife can also be used to remove diatoms from the substratum and then rinsed in some stream water collected directly into a sample bottle, if this is preferred.

If > 75 % of substrata are smothered with filamentous algae, these should be sampled in preference to substrata lacking such growths. Remove as many of the filaments as possible prior to brushing or scraping, as above.

Replace the substratum in the stream, and repeat the process for the other replicate substrata. Transfer the water, which should now be brown and turbid due to the presence of diatoms, from the tray into the sample jar.

Label the sample bottle with details relevant to the sample. Transfer the sample to the laboratory in a cool, dark place. If samples are brought to the laboratory within 24 h and these precautions are followed, it is not necessary to add preservative in the field. If preservatives are necessary, then these should be added immediately after collection, unless there are other reasons (e.g. health and safety) why preservatives cannot be used in the field. All future handlers of preserved samples shall be informed of the nature of any preservatives present.

6.3.2 Method for sampling vertical man-made surfaces *in situ*

The criteria listed above for microhabitat selection should be followed as far as possible.