SLOVENSKI STANDARD
oSIST prEN 13946:2024

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Kakovost vode - Navodilo za rutinsko vzorčenje in pripravo vzorcev bentoških kremenastih alg rek in jezer

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

Wasserbeschaffenheit - Anleitung zur Probenahme und Probenaufbereitung von benthischen Kieselalgen aus Fließgewässern und Seen

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

Ta slovenski standard je istoveten z: **prEN 13946**

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13.060.10	Voda iz naravnih virov	Water of natural resources
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English Version

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

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

Wasserbeschaffenheit - Anleitung zur Probenahme und Probenaufbereitung von benthischen Kieselalgen aus Fließgewässern und Seen

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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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European foreword

This document (prEN 13946:2024) 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 will supersede EN 13946:2014.

This document (prEN 13946:2024) includes the following significant technical changes with respect to EN 13946:2014:

— the method has been adapted to process the samples obtained for subsequent molecular methods by adding additional solvents and requirements to avoid contamination of samples.

Introduction

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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 ecological status or the impact of specific components of water quality (e.g. eutrophication, acidification). The method is appropriate for assessments required by the Water Framework Directive (2000/60/EC) and Urban Waste Water Treatment Directive (91/217/EEC) in addition to other EU Directives and international agreements. This document covers aspects of sampling and preparation relevant to assessment of water quality and ecological status using benthic diatoms. These instructions will result in samples suitable for quantifying relative numbers of benthic diatom taxa present using either light microscopy or molecular methods. 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 document.

The use of diatoms as indicators of river and lake quality is widely accepted both in Europe and beyond. The methodology is based on observations that all diatom species have distinct preferences for particular 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 may 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 references [1] to [4]). The methods for evaluating the data vary but the sampling and preparation processes are similar [5, 6]. In recent years, molecular methods such as metabarcoding have been developed to the point where they are suitable for routine use [7, 8] and modifications to procedures appropriate for this approach have been included in this revision of the standard.

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

All numerical values given in this document are approximate.

1 Scope

This document specifies a method for the sampling and laboratory preparation of benthic diatoms for ecological status and water quality assessments. The sampling and preparation procedures described can be used for later investigations using either light microscopy or molecular methods. Data produced by this method are suitable for production of indices based on the relative abundance of taxa.

Analysis using molecular methods is not within the scope of the document.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp/

— IEC Electropedia: available at https://www.electropedia.org/

3.1
benthic diatoms
diatoms living on natural or artificial substrata, rather than suspended in the water column

3.2
boulder
mineral substratum with a diameter > 256 mm

3.3
cobble
mineral substratum with a diameter > 64 mm and ≤ 256 mm

3.4
ecological status
measure of the structure and functioning of aquatic communities

3.5
euphotic zone
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
specific environment in which an organism lives

3.8
introduced substrata
artificial substratum
substratum introduced into river or lake by operator specifically for colonisation by diatoms

3.9
metabarcoding
identification (and quantification) of many species from a single sample based on sequences from a short DNA/RNA region

3.10
molecular method
method that use molecular genetic technologies in order to elucidate the properties of biological samples

3.11
pebble
mineral substratum with a diameter > 16 mm ≤ 64 mm

3.12
riffle
shallow part of a stream with swift flow, usually with a broken surface

3.13
substratum
natural or non-natural material from which benthic diatoms are sampled

3.14
taxa
taxonomic units, for example families, genera or species

3.15
valve
structural component of the diatom frustule (3.6)

4 Principle

Benthic diatoms from submerged hard surfaces or submerged macrophytes in rivers, streams or littoral zones of lakes are sampled in order to produce representative collections of the diatom assemblage indicative of ecological status and water quality. Samples are cleaned using strong oxidizing agents in order to prepare diatoms for identification and enumeration, or preserved for analysis by molecular methods.

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

5 Equipment

5.1 Field sampling

Apparatus and equipment used for removing biofilms from surfaces shall be free from any contamination that may bring diatoms or DNA traces from other samples. It is not necessary to use a brand new toothbrush for every site, but a strict cleaning procedure after use is recommended to avoid cross contamination [9, 10]. Toothbrushes can be cleaned by rinsing and rubbing to remove coarse materials. When samples are to be used for metabarcoding, stronger cleaning agents (e.g. a 10 % bleach solution for 10 min) should be used before thoroughly rinsing and drying.

5.1.1 Appropriate water safety equipment.

5.1.2 Waders

5.1.3 Toothbrush with stiff bristles (or other similar instrument) **or knife** (or other suitable blade).

5.1.4 Plastic tray (approximately 30 cm × 20 cm or larger).

5.1.5 Sample bottle with a tight fitting lid.

5.1.6 Indelible marker pen or other means of labelling samples.

If labels are used, these should be capable of surviving wet conditions.

5.1.7 Hoe, with a fine-meshed net attached, attached to long handle if vertical hard surfaces are to be sampled.

5.1.8 A glass-bottomed box or bucket ("Aquascope"/"Hydroscope").

Useful for finding suitable substrata under some circumstances.

5.2 Laboratory equipment

See Annex A.

NOTE Laboratory methods for light microscopy only are included in this document.

6 Reagents

6.1 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.

6.2 Preservatives

These are required to stop cell division of diatoms and decomposition of organic matter or, in the case of samples collected for analysis by metabarcoding, degradation of their DNA. No preservative is necessary if the sample is to be processed for light microscopy 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 prior to analysis by light microscopy; 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 for light microscopy may also be frozen.

For metabarcoding, Lugol's iodine and formaldehyde cannot be used because they do not adequately preserve environmental DNA [11]. Ethanol or a nucleic acid preservative is recommended for the preservation and storage of samples for analysis by metabarcoding [12]. Samples may also be frozen (as centrifuged pellets) immediately on return from the field.

6.2.1 Buffered 4 % (minimum volume fraction) 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 hexamethylene-tetramine.

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

NOTE The buffer is necessary to prevent dissolution of the silica frustules. This is only necessary in alkaline waters, as diatoms dissolve in alkaline water.

6.2.2 Lugol's iodine

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

Add 1 drops 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.

6.2.3 Ethanol (C_2H_5OH)

A final concentration of 20 % is recommended for medium-term storage for light microscopy.

A final concentration of at least 70 % is recommended for storage for molecular methods.

70 % ethanol has been shown to be suitable for storage for periods of up to a year priori to analysis by molecular methods [12]. For longer-term storage, higher concentrations may be appropriate. Molecular-grade ethanol should be used for this purpose.

6.2.4 Hydrochloric acid (HCl)

10 % hydrochloric acid can also be used for medium-term storage for light microscopy. By adding acid, the diatoms detach from their substrata and iron- and calcium-complexes will dissolve.

6.2.5 Freezing

Immediate freezing to −20 °C or below is recommended for preservation and storage of samples intended for analysis by molecular methods.

6.2.6 Other preservatives for metabarcoding

Nucleic acid preservatives are suitable for storing samples for analysis by molecular methods. For example, a mixture of 3,5 M ammonium sulphate, 17 mM sodium citrate and 13 mM ethylenediaminetetraacetic acid (EDTA) (sold commercially as "RNAlater") can be used, so long as the sample is frozen immediately on return to the laboratory.

6.3 Reagents for cleaning diatoms for light microscopy

See Annex A.

6.4 Reagents for preparing permanent slides

A diatom mountant with a refractive index > 1,6 is required (e.g. Naphrax).

7 Sampling procedure

7.1 Choice of substratum

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

Areas of the riverbed or lake littoral zone 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 also 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 may also be sampled, if these have been in the river or lake 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.