



SLOVENSKI STANDARD

SIST EN 13946:2014

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Nadomešča:
SIST EN 13946:2003

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

Water quality - Guidance 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

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EUROPEAN STANDARD

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English Version

Water quality - Guidance 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

This European Standard was approved by CEN on 20 December 2013.

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Foreword

This document (EN 13946:2014) 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of an identical text or by endorsement, at the latest by September 2014, and conflicting national standards shall be withdrawn at the latest by September 2014.

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.

This document supersedes EN 13946:2003.

This document contains the following technical changes in comparison with the previous edition:

- this European Standard is now also applicable for the sampling of benthic diatoms in lakes.

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, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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Introduction

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 based on diatoms as one compartment of the ecosystem or the impact 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) [7] and Urban Waste Water Treatment Directive (91/271/EEC) [8] in addition to other EU Directives and international agreements. This European Standard covers aspects of sampling and preparation relevant to assessment of water quality and ecological status using benthic diatoms. 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 European Standard.

The use of diatoms as indicators of river and lake 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 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 the proceedings of three symposia [1] to [3]). The methodologies for evaluating the diatom data vary but the sampling and preparation processes are similar [5, 6].

According to the precise usage to which this European 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 standard are approximate.

WARNING — Persons using this European Standard should be familiar with usual laboratory 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 user to establish appropriate health and safety practices and to ensure compliance with any national regulatory conditions.

1 Scope

This European Standard specifies a method for the sampling and laboratory preparation of benthic diatoms for ecological status and water quality assessments. Data produced by this method are suitable for production of water quality indices based on the relative abundance of taxa.

2 Terms and definitions

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

2.1

artificial substratum

introduced substratum

substratum introduced into river or lake by operator specifically for colonization by diatoms

2.2

benthic diatoms

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

2.3

boulder

mineral substratum with a diameter > 256 mm

2.4

cobble

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

2.5

ecological status

measure of the structure and functioning of aquatic communities

2.6

euphotic zone

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

2.7

frustule

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

2.8

habitat

specific environment in which an organism lives

2.9

pebble

mineral substratum with a diameter > 16 mm and ≤ 64 mm

2.10

riffle

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

2.11

substratum

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

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EN 13946:2014 (E)**2.12****taxa**

taxonomic units, for example families, genera or species

2.13**valve**

structural component of the diatom frustule

3 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 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 [1], [2], and [3]).

4 Equipment**4.1 Equipment for field sampling****4.1.1 Appropriate water safety equipment****4.1.2 Waders****4.1.3 Stiff toothbrush** (or other similar instrument) or **knife** (or other suitable blade).**4.1.4 Plastic tray** (approximately 30 cm × 20 cm or larger).**4.1.5 Sample bottles** with a tight fitting lid.**4.1.6 Indelible marker pen** (or other means of labelling samples).

If labels are used, these shall be capable of withstanding wet conditions.

4.1.7 Hoe, with a fine-meshed net attached, attached to long handle (if vertical hard surfaces are to be sampled).**4.1.8 Glass-bottomed box or bucket** ("Aquascope") used for finding suitable substrata under some circumstances.**4.1.9 Plastic bags****4.2 Laboratory equipment**

See Annex A.

5 Reagents

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.

5.1 Preservatives

Preservatives 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 may also be deep-frozen.

5.1.1 Buffered formaldehyde solution, HCHO, volume fraction at least 4 %.

Dilute a stock solution of formaldehyde to a volume fraction of 4 % in a solution buffered to pH 7. Suitable buffers include HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), borate and hexamethylene-tetramine.

A final solution with a volume fraction of 1 % to 4 % in the sample 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. This is only necessary in alkaline waters, as diatoms dissolve in alkaline water.

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

5.1.3 Ethanol, C₂H₅OH, volume fraction 70 %.

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

5.1.4 Diluted hydrochloric acid, HCl, volume fraction 10 %.

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

5.2 Reagents for cleaning diatoms

See Annex A.

5.3 Reagents for preparing permanent slides

A diatom mountant with a refractive index > 1,6 is required (e.g. Naphrax¹).

1) Naphrax is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.