SLOVENSKI STANDARD
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Kakovost vode - Tehnično poročilo o rutinskem vzorčenju bentoških kremenastih alg v rekah in jezerih, prilagojeno za analize metabarkodiranja

Water quality - Technical report for the routine sampling of benthic diatoms from rivers and lakes adapted for metabarcoding analyses

Wasserbeschaffenheit - Technischer Bericht zur Routine-Probenahme von benthischen Diatomeen in Flüssen und Seen für Metabarcode-Analysen

Qualité de l'eau - Rapport technique concernant l'échantillonnage de routine de diatomées benthiques dans les rivières et les lacs adapté aux analyses reposant sur l'utilisation de métacodes à barres d'ADN

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TECHNICAL REPORT

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Water quality - Technical report for the routine sampling of benthic diatoms from rivers and lakes adapted for metabarcoding analyses

Qualité de l'eau - Rapport technique pour l'échantillonnage en routine de diatomées benthiques dans les rivières et les plans d'eau adapté pour les analyses en metabarcoding

Wasserbeschaffenheit - Technischer Bericht zur routinemäßigen Beprobung benthischer Diatomeen aus Fließgewässern und Seen angepasst für Metabarcodinganalysen

This Technical Report was approved by CEN on 14 May 2018. It has been drawn up by the Technical Committee CEN/TC 230.

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European foreword

This document (CEN/TR 17245:2018) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

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Introduction

WARNING — Persons using this document should be familiar with normal laboratory practice. This technical report 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 unicellular microalgae present in all types of water bodies. They are an important component of aquatic ecosystems and have been used widely for ecological assessment 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. The use of diatoms as indicators of river and lake quality is based on observations that diatom taxa have distinct preferences for particular environmental conditions such as nutrients, organic pollution and acidity. Polluted waters will tend to support higher proportions of those taxa 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 and ecological status based on optical microscopy have been developed in several European countries. Methods for sampling and preparation are similar [6], [7] leading to the development of European Standards which, in turn, facilitated the harmonization of ecological assessment approaches [2], [3]. More recently, however, molecular biology has presented new opportunities for assessment of ecological status using diatoms e.g. [4], [5], [13]. Such procedures, based on metabarcoding methodologies, however, are not covered by existing standards.

Methods using diatoms to assess water quality have been developed in several European countries (summarized in [8], [10], [11], [12]). The methodologies for the ecosystem assessment vary but the sampling is similar [6], [7]. Usually sample preparation is done for subsequent microscopic analyses. This technical report is a supplement of EN 13946 "Water quality — Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes" and gives the requirements for subsequent metabarcoding analyses.

According to the precise usage to which this technical report 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 technical report are approximate.

1 Scope

This technical report specifies a method for the field sampling of benthic diatoms which will be then analysed by subsequent metabarcoding techniques for ecological status and water quality assessments. Data produced by this method are suitable for production of taxonomical diatom lists.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 13946:2014, *Water quality — Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 13946:2014 and the following apply.

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

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

3.1
barcode
stretch of a DNA sequence of at least 100-400 base pairs length that uniquely identifies a specific taxon

3.2
base pair
pair of nucleotides that is the building block of the DNA double helix

3.3
environmental DNA
DNA that can be extracted from air, water, or soil, without isolating any specific type of organism beforehand

4 Principle

4.1 General

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. Field sampling is the same as EN 13946:2014, specifications for field sample fixation adapted for metabarcoding are given. When specifications are identical from EN 13946:2014, only a reference about the EN 13946:2014 paragraph is given. Metabarcoding analyses, which will enable to produce taxonomical diatom list will be given in separated standards or technical report.

4.2 Equipment for Field sampling

See EN 13946:2014, 4.1 for the equipment list. In addition: sample bottle with a tight fitting lid shall be sterile and free of DNA traces.

5 Reagents

5.1 General

Reagents used for sample preservation and sample bottle need to be free of DNA traces. Reagents should be of appropriate quality.

5.2 Preservatives

5.2.1 General

Preservatives are required to stop cell division of diatoms, decomposition of organic matter and preservation of environmental DNA. Ethanol or deep-freezing are recommended. Alternative methods for environmental DNA preservation exist but are difficult to use for routine field work. Lugol's iodine and formaldehyde cannot be used because they do not adequately preserve environmental DNA [1].

5.2.2 90 % Ethanol (C_2H_5OH)

A final concentration of 70 % is required for preservation and sample storage and its environmental DNA.

5.2.3 Deep-freezing

Deep-freezing -20 °C or below is required for preservation and sample storage and its environmental DNA.

5.2.4 Other commercial preservatives

Other aqueous solution adapted to DNA or RNA preservation that can be purchased commercially can be used after showing their efficiency.

6 Procedure

6.1 Choice of substratum

In accordance with EN 13946:2014, 6.1.

6.2 Sample site selection

In accordance with EN 13946:2014, 6.2.

6.3 Sampling methods

6.3.1 Moveable natural hard surfaces

Follow instructions of EN 13946:2014, 6.3.1. The following instructions for equipment cleanliness shall be followed:

Use a clean stiff toothbrush free of any DNA traces bringing contamination from other samples. Brush the upper surface of the substratum vigorously to remove the diatom film, rinsing the toothbrush periodically in the water.

A knife or other sharp instrument may also be used to remove the diatom film. These instruments shall not be contaminated by DNA traces coming from other samples. Add preservatives immediately after sampling (6.4).

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

See EN 13946:2014, 6.3.2.

For details regarding labelling and sample preservation see 6.3.1 and 6.4.

6.3.3 Use of introduced ("artificial") substrata

See EN 13946:2014, 6.3.3. For details regarding labelling and sample preservation see 6.3.1 and 6.4.

6.3.4 Sample collection from submerged macrophytes and macroalgae

See EN 13946:2014, 6.3.4. In each case label the bottle (see 6.3.1) and preserve the sample immediately (see 6.4).

6.3.5 Sample collection from emergent macrophytes

See EN 13946:2014, 6.3.5.Then label the bottle (see 6.3.1) and preserve the sample immediately (see 6.4).

6.4 Sample preservation

6.4.1 Ethanol preservation

Ethanol shall be added to the sample immediately after sampling in the field. A final concentration in the sample bottle of 70 % is required for preserving and storing the sample and its environmental DNA (Kermarrec 2012, Kermarrec et al. 2014).

6.4.2 Deep-freezing preservation

Just after sampling, the sample bottle shall be stored in a cooling box or in a mini-refrigerator in the field (temperature around 7 °C). Deep-freezing at -20 °C shall be carried out within a few hours (less than 4 h to 5 h).

6.4.3 Other commercial preservatives

Commercial solutions adapted to DNA or RNA preservation shall be added immediately after sampling in the field. Final concentration in the sample shall be adapted to the quantity of biofilm sampled. The amount of preservative solution recommended by the manufacturer shall be checked during initial use and validated before routine use.