



BSI Standards Publication

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

---

## National foreword

This Published Document is the UK implementation of CEN/TR 17245:2018.

The UK participation in its preparation was entrusted to Technical Committee EH/3, Water quality.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

© The British Standards Institution 2018  
Published by BSI Standards Limited 2018

ISBN 978 0 580 52468 4

ICS 13.060.70

**Compliance with a British Standard cannot confer immunity from legal obligations.**

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 30 September 2018.

### Amendments/corrigenda issued since publication

Date	Text affected
------	---------------

---

TECHNICAL REPORT

**CEN/TR 17245**

RAPPORT TECHNIQUE

TECHNISCHER BERICHT

September 2018

ICS 13.060.70

English Version

## 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.

CEN members are the national standards bodies of 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, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



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

**CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels**

<b>Contents</b>	<b>Page</b>
European foreword.....	3
Introduction .....	4
1 Scope .....	5
2 Normative references .....	5
3 Terms and definitions .....	5
4 Principle .....	5
4.1 General .....	5
4.2 Equipment for Field sampling .....	5
5 Reagents .....	6
5.1 General .....	6
5.2 Preservatives .....	6
5.2.1 General .....	6
5.2.2 90 % Ethanol (C <sub>2</sub> H <sub>5</sub> OH) .....	6
5.2.3 Deep-freezing .....	6
5.2.4 Other commercial preservatives .....	6
6 Procedure .....	6
6.1 Choice of substratum .....	6
6.2 Sample site selection .....	6
6.3 Sampling methods .....	6
6.3.1 Moveable natural hard surfaces .....	6
6.3.2 Method for sampling vertical man-made surfaces <i>in situ</i> .....	6
6.3.3 Use of introduced (“artificial”) substrata .....	7
6.3.4 Sample collection from submerged macrophytes and macroalgae .....	7
6.3.5 Sample collection from emergent macrophytes .....	7
6.4 Sample preservation .....	7
6.4.1 Ethanol preservation .....	7
6.4.2 Deep-freezing preservation .....	7
6.4.3 Other commercial preservatives .....	7
Bibliography .....	8

## **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.

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

## **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 (91/271/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.