



SLOVENSKI STANDARD

SIST EN 14407:2005

01-februar-2005

Kakovost vode - Navodilo za identifikacijo, štetje in razlago vzorcev bentoških kremenastih alg vodotokov

Water quality - Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters

Wasserbeschaffenheit - Anleitung zur Bestimmung, Zählung und Interpretation von benthischen Kieselalgen in Fließgewässern

Qualité de l'eau - Guide pour l'identification et le dénombrement des échantillons de diatomées benthiques de rivières, et leur interprétation

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ICS:

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
NORME EUROPÉENNE
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EN 14407

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English version

Water quality - Guidance standard for the identification,
enumeration and interpretation of benthic diatom samples from
running waters

Qualité de l'eau - Guide pour l'identification et le
dénombrement des échantillons de diatomées benthiques
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Fließgewässern

This European Standard was approved by CEN on 21 May 2004.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

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 Central Secretariat has the same status as the official versions.

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EN 14407:2004 (E)

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Foreword

This document (EN 14407:2004) 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 February 2005, and conflicting national standards shall be withdrawn at the latest by February 2005.

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

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Introduction

WARNING — Persons using this 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 specific components of water quality (e.g. eutrophication, acidification). The requirement for the monitoring of such processes is inherent in the Urban Wastewater Treatment Directive (91/271/EEC) and the Water Framework Directive (2000/60/EC) in addition to other EU Directives and international agreements. This standard covers aspects of identification and enumeration of the relative abundance of diatom taxa on prepared slides and of data interpretation relevant to assessment of water quality.

The use of diatoms as indicators of the quality of river waters is widely accepted both in Europe and the USA. The methodology is based on the fact that all diatom species have optima with respect to their tolerance (or preference) for environmental conditions such as nutrients, organic pollution, pH etc. Polluted waters will tend to support an increased abundance of those species whose optima correspond with the levels of the pollutant in question.

Methods using diatoms to assess water quality have been developed in several European countries (recent work is summarised in the proceedings of three symposia (references [1] to [3])).

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. It should be noted that some indices and national methodologies have particular requirements whilst this document embraces the full range of valid options. Under such circumstances, the requirements of a particular index should take priority.

1 Scope

This document establishes methods for the identification and enumeration of relative proportions of diatom taxa on prepared slides and of data interpretation relevant to assessments of water quality in rivers and streams. It is suitable for use with indices and assessment methods based on the relative abundance of taxa. The methods for identification and enumeration can also be applied to the study of benthic diatoms in other habitats provided that data interpretation methods appropriate to these habitats are used.

2 Normative references

The following referenced documents are indispensable for the application 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, *Water quality - Guidance standard for the routine sampling and pretreatment of benthic diatoms from rivers*

3 Terms and definitions

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

3.1

benthic diatoms

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

3.2

expected natural community

community present at a site when only natural stresses (e.g. floods) occur and man-made stress is not significant

3.3

eyepiece graticule

measuring device, inserted into one eyepiece of a microscope, permitting absolute measurements of the size of objects. The relationship between each division on the eyepiece graticule and the actual size of the object will depend upon the magnification of the microscope

3.4

frustule

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

3.5

habitat

specific environment in which an organism lives

3.6

prepared slide

slide plus coverslip on which has been mounted a sub-sample of diatoms in accordance with criteria outlined in EN 13946

3.7

stage micrometer

slide on which is inscribed a known distance, typically divided into sub-divisions, against which an eyepiece graticule can be calibrated

EN 14407:2004 (E)**3.8****taxon (pl. taxa)**

taxonomic units, for example families, genera or species

3.9**unit**

terminology adopted in this document to refer to either diatom valves or intact frustules, depending upon the conventions adopted in 6.2

3.10**valve**

structural component of the diatom frustule (see above)

3.11**vernier scale**

measuring device associated with the mechanical stage of a microscope, permitting the relative transverse and longitudinal position of a slide to be noted with a typical precision of about 0,1 mm

3.12**watercourse**

body of surface water that has running water perennially or at some time during the annual hydrologic cycle

4 Principle

Benthic diatoms, cleaned of cell contents and mounted in a medium with a high refractive index, are identified and counted using a high power light microscope until an appropriate sample size has been obtained. These data are then interpreted using one or more indices or other assessment methods.

5 Requirements

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- Light microscope, equipped with a mechanical stage and high power (e.g. 100× magnification) oil-immersion lens. Use of a phase contrast or differential interference (Nomarski) condenser is recommended. The microscope should incorporate facilities for measurements (e.g. an eyepiece graticule) with a resolution of at least 1 µm. Apparatus for photomicroscopy or video capture are useful for documentation of difficult specimens and can also assist with measurement of striae density etc.
- Floras, identification guides and iconographs, appropriate to the habitats under consideration (see below).
- Immersion oil and dispenser
- Lens tissue
- Facility for recording data as they are collected. This can be a *pro forma* count sheet with a list of taxon names and space beside each on which the counts can be made or a laboratory notebook organised in such a way that taxon identities and numbers can be clearly recorded, or a computer program with facilities for direct entry of data.

NOTE The design of the count sheets or programs should take into account the requirements of any Quality Assurance programmes that are in place.

- Facility for verifying the identity of difficult specimens. This can take several forms: drawings, high quality photomicrographs or captured video images may suffice. However, it is also useful to be able to relocate actual specimens. If taxonomic assistance is available "in house", noting co-ordinates on the microscope's Vernier scale may be sufficient. If another microscope is likely to be used, then a facility to record the absolute position of the specimen may be necessary.

6 Preliminary stages

6.1 Determining taxonomic criteria for analysis

Recent debates about the fundamentals of diatom taxonomy have led to the co-existence of parallel systems of nomenclature. It is important, when using diatoms for water quality assessments to ensure that any scope for confusion about the correct name to apply to a diatom is eliminated.

The minimum level of taxonomy that is acceptable for a study will be determined by the intended uses of the data. Most pollution indices require species-level identification, although some can be used with genera, or a mixture of genera and species.

Adopting the nomenclature of a comprehensive flora relevant to the study area is recommended; however, it is also possible to use a national or regional checklist of diatoms. When taxonomic conventions of the index and the checklist differ, the conventions of the index should be adjusted to those of the checklist. This should be done in advance and the correct nomenclature recorded in standard operating procedures. Taxonomic authorities should be cited in all cases where there is any potential for nomenclatural confusion.

6.2 Determining units for enumeration

Different conventions have evolved for enumeration of diatoms, using either valves or frustules as the basic unit, or not distinguishing between valves and frustules. The effect that such conventions have on final results has not been formally evaluated but experts believe that it is likely to be small. However, it is important that the convention is specified in advance. In the case of small diatoms, such as some *Achnanthes* and *Navicula* species, it may not be possible to distinguish between intact frustules and isolated valves with certainty on all occasions.

6.3 Determining sample size

The number of units necessary to compute diatom-based pollution indices will depend upon the uses to which the data are put. A typical count size is 300 to 500 units, although lower or higher numbers may be appropriate for some purposes. Lower numbers may lack the statistical rigour necessary for some applications. The minimum and maximum number of units should be appropriate to the objectives of the study and should be specified in advance. Standard operating procedures should also specify situations under which alternative procedures, such as stratified counting protocols (see clause 7h), should be used. Likely situations include cases where the sample is overwhelmingly dominated by a cosmopolitan taxon, or by a taxon not included in the index calculation. In such situations, the target count size should be applied only to those taxa that are included in any relevant calculations although taxa not included in index calculations should be counted as well, as these may still contribute information useful for interpreting index results. Details of studies with a range of objectives may be found in references [1] to [3].

6.4 Preparation of the microscope

The eyepiece graticule, or other measuring equipment, should be calibrated against a stage micrometer regularly. The results of this calibration should be displayed in a position where users of the microscope can consult them. A resolution of 1µm is adequate for routine analyses.

The second eyepiece may be equipped with a second graticule to aid enumeration. This can take several forms: including a square grid, H-shape, Whipple field etc. The important point is that this is linked to a "house rule" that ensures that no diatom is counted more than once. Options for enumeration are:

- 1) A slow vertical or horizontal traverse is performed, with each diatom identified and added to the total as it passes one of the lines on the eyepiece graticule; or,
- 2) All diatoms visible in a field of view (or within the grid of a graticule) are identified and counted before **either** moving along a horizontal or vertical traverse to the next field **or** selecting a new field of view at random.