SLOVENSKI STANDARD
SIST EN 15708:2010

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Kakovost vode - Smerni standard za rutinsko pregledovanje, vzorčenje in laboratorijske analize fitobentov v plitvih vodotokih

Water quality - Guidance standard for the surveying, sampling and laboratory analysis of phytobenthos in shallow running water

Wasserbeschaffenheit - Anleitung zur Beobachtung, Probenahme und Laboranalyse von Phytobenthos in flachen Fließgewässern

Qualité de l'eau - Guide pour l'étude, l'échantillonnage et l'analyse en laboratoire du phytobenthos dans les cours d'eau peu profonds

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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

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English Version

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COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 15708:2009) 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of an identical text or by endorsement, at the latest by May 2010, and conflicting national standards shall be withdrawn at the latest by May 2010.

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Introduction

WARNING — Working in or around water is inherently dangerous. Persons using this European Standard should be familiar with normal laboratory practice. Long periods of analysis at the microscope can cause physical fatigue and affect eyesight. Attention should be given to the ergonomics of the microscope and advice from a health and safety practitioner should be sought to ensure that risks are minimized. The use of chemical products mentioned in this standard can be hazardous and users should follow guidelines provided by the manufacturers and take necessary specialist advice. This standard does not purport to address the safety problems 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.

The phytobenthos is an important component of aquatic ecosystems and an understanding of the composition of the phytobenthos present in a waterbody can provide useful information on the status of that waterbody, and on appropriate management strategies. The Water Framework Directive (2000/60/EC) [3] requires monitoring of the phytobenthos as a quality element used for ecological status assessment, and phytobenthos assessments have also been used in monitoring programmes associated with other European Directives (e.g. Urban Wastewater Treatment Directive, Habitats Directive) and with national legislation (e.g. ÖNORM M6231).

This guidance standard specifically relates to the sampling of phytobenthos (other than aquatic macrophytes) in running water. An etymologically-correct application of the term "phytobenthos" would cover all phototrophic organisms; however, this encompasses a vast range of organisms, from microscopic unicells to macrophytes > 2 m in length. As separate survey methods for aquatic macrophytes are available (EN 14184), this document focuses on phototrophic algae and oxygenic cyanobacteria that live on substrata. Bryophytes are common in shallow running waters and competitive interactions between these and larger algae are common. Similarly, aquatic macrophyte species may, themselves, act as substrata or competitors for algae and cyanobacteria. For these reasons, the standard provides options for including these taxa in survey and sampling procedures. The term "periphyton" is sometimes used instead of "phytobenthos"; however, some definitions of "periphyton" include heterotrophic organisms that live attached to substrata (protozoa, sponges, hydroides). Methods described here deal only with photosynthetic organisms but they could, if required, be adapated to encompass heterotrophic organisms too.

Methods using phytobenthos to assess water quality in running water have been developed in several European countries [6], [8], [9], [10] and in the USA [2]. Recent work is summarised in the proceedings of four symposia [1], [7], [11], [12]. Methods for the sampling and analysis of one group of phytobenthos, the diatoms, have already been the subject of harmonisation (EN 13946, EN 14407). However, these standards are concerned with only a single group of the phytobenthos and there are situations where other phototrophs are more obvious and can contribute additional ecological information.

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.

1 Scope

This European Standard provides guidelines for the survey/sampling, identification and basic quantification of phytobenthos (other than macrophytes) in running waters. It is applicable to rivers where benthic algae and bryophytes are the main phototrophs. This method encompasses all phytobenthic growth forms and enables biological responses to environmental events over one or more years to be monitored. In this respect it provides an alternative to methods based on benthic diatoms (EN 13946; EN 14407) and macrophytes (EN 14184). Data obtained for the phytobenthos growth forms are suitable for pilot surveys, water quality assessment and trend monitoring. This European Standard encompasses all aspects from the design of survey and sampling programmes to the identification and basic quantification of the phytobenthos.

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*

EN 14407, *Water quality — Guidance standard for the identification, enumeration and interpretation of benthic diatom samples from running waters*

EN 15204, *Water quality — Guidance standard on the enumeration of phytoplankton using inverted microscopy (Utermöhl technique)*

3 Terms and definitions

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

3.1
aquatic macrophytes
larger plants of fresh water which are easily seen with the naked eye, including all aquatic vascular plants, bryophytes, stoneworts (Characeae) and macro-algal growths

[EN 14184:2003, 3.1]

3.2
assemblage
organisms that share a sampling area

NOTE This term is preferred to “community”, as the latter implies a level of ecological integration of the organisms; whereas sampling may inadvertently combine representatives from more than one true “community” that are not distinct to the naked eye.

3.3
belt transect
defined band across a river or stream at right angles to the bank

NOTE This may be virtual or physically delineated within which the aquatic vegetation is analysed (species composition, abundance, cover).

[EN 14184:2003, 3.4]

3.4
benthic alga
alga or oxygenic cyanobacterium living attached to a substratum (rather than suspended in the water column)

3.5
biofilm
mucilagineous polysaccharide matrix on submerged stable surfaces consisting of photo(auto)trophic and heterotrophic organisms

3.6
boulder
mineral substratum with a diameter > 256 mm

[EN 13946:2003, 3.3]

3.7
bryophyte
collective term for liverworts and mosses – plants which are often abundant on boulders and bedrock of fast flowing streams

3.8
cobble
mineral substratum with a diameter > 64 mm and ≤ 256 mm

[EN 13946:2003, 3.4]

3.9
degree of cover
percent of substratum at the sampling site covered (by the organism)

3.10
epilithic alga
alga living attached to or in close association with a stony substratum

3.11
epiphytic alga
alga living attached to or in close association with macrophytes or other algae

3.12
epipelic alga
alga that lives in or on fine sediments

3.13
epipsammic alga
alga that lives attached to or in close association with sand

3.14
habitat
specific environment in which a species lives

[EN 15460:2007, 3.5]

3.15
macroscopic benthic alga
multicellular alga or an aggregation (units/groupings) of unicellular algae living attached to substrata that is visible to the naked eye

3.16
nuisance biomass
accumulations of benthic algae that are a nuisance to users of the watercourse and/or that detrimentally affect its ecology

3.17
periphyton
group of organisms (principally algae, but also including fungi, bacteria and protozoa) living on or in close contact with surfaces in aquatic environments

NOTE 1 Bryophytes have an intermediate position. They are often regarded as a component of the aquatic macrophytes, particularly in slow flowing rivers where aquatic macrophytes are common.

NOTE 2 The term “periphyton” is often used as a synonym for benthic algae in recent literature.

3.18
phototroph
organism whose main source of carbon is obtained through photosynthesis

NOTE For the purpose of this document, facultative phototrophs such as many Euglenophyta are included within this definition.

3.19
phytobenthos
all phototrophic algae and oxygenic cyanobacteria living on or in close contact with surfaces in aquatic environments

NOTE This term is virtually synonymous with the term "periphyton", although some people also include heterotrophic organisms in definitions of the latter.

3.20
reach

length of a watercourse forming a major sub-division of a river basin and defined by physical, chemical or hydrological characteristics (or any combination of these) that distinguishes it from the watercourse upstream and downstream

NOTE The boundaries between reaches mark the principal points of transition where the overall character of the watercourse changes.

[EN ISO 8689-2:2000, 3.1]

3.21
reference conditions
conditions reflecting a totally undisturbed state, or one with only very slight human impacts, or near-natural with only minor evidence of distortion

3.22
riffle
fast-flowing shallow water with distinctly broken or disturbed surface over gravel/pebble or cobble substratum

[EN 14614:2004, 2.28]

3.23
survey unit
length of river from which data are collected during field survey; this may be a fixed length (e.g. 10 m) or variable, according to the methods used, but must always be defined and recorded

[Adapted from EN 14614:2004, 2.38]

3.24
taxon
group of organisms related at a particular taxonomic level

[EN 14996:2006, 3.20]

NOTE Plural is "taxa".

4 Principle

Phototrophs associated with submerged surfaces in running water are surveyed and/or sampled. Specimens of those taxa that cannot be identified in the field are taken back to the laboratory for identification. Three different options are provided within the standard, suitable for different circumstances. Outcomes of the survey/sampling process may include:

a) a list of all macroscopic algae (and, optionally, non-vascular plants) observed in the survey unit;

b) a list of all macroscopic and microscopic algae (and, optionally, non-vascular plants) observed in the survey unit; or

c) a list of all microscopic and macroscopic algae found on a single substratum within the survey unit.

Semi-quantitative estimates of the abundance of each taxon are also made. These data can be used to give an integrated picture of ecological status and/or water quality.

5 Reagents

Preservatives are necessary if samples are to be stored prior to analysis. If treated with care, many algal samples can be stored in a refrigerator or cool room for several days without deterioration. However, where long-term storage is necessary, then a preservative may be necessary. Recipes for preparation of these are given in EN 15204 (see also 8.4).

6 Equipment

6.1 Field equipment

6.1.1 Necessary field equipment

6.1.1.1 Appropriate water safety equipment

6.1.1.2 A means of locating sampling reaches on repeat visits, if there is no permanent landmark adjacent. Options include iron bolts, fast drying paint, waterproof tape or similar to delimit the sampling reaches.

6.1.1.3 Waders

6.1.1.4 Aqua-scope, or bucket with clear Perspex base, for scanning the river bottom in turbulent water.

6.1.1.5 Stainless steel knife or other suitable blade, forceps and stiff toothbrush

6.1.1.6 Hand lens

6.1.1.7 White plastic or enamel tray, volume 2 l to 3 l, for sorting material and sub-sampling.

6.1.1.8 **Sample vials with tight fitting lids**, recommended sizes are 5 ml and 125 ml, to encompass both singular macroscopic units and composite samples.

6.1.1.9 **Waterproof labels for sample vials**, or a marker pen with waterproof ink.

6.1.1.10 **Waterproof fieldbook**, or standardised recording sheets plus pencil or indelible pen.

6.1.1.11 **Preservative**, buffered formalin, Lugol's iodine, or other.

CAUTION — The use of formalin can cause health problems.

6.1.2 Optional field equipment

6.1.2.1 **Global positioning system (GPS) receiver**

6.1.2.2 **Rake with attached net** or hoe attached to a long handle, to facilitate sampling at high flow.

6.1.2.3 **Bucket**, to transfer large substrata to laboratory.

6.1.2.4 **Camera or video-camera**

6.1.2.5 **Portable refrigerator or ice box**

6.1.2.6 **Boxes with room**, to store all sample vials from one locality, to facilitate storage.

6.2 Laboratory equipment

6.2.1 Necessary laboratory equipment

6.2.1.1 **Binocular microscope**, equipped with a mechanical stage and at least 40 × magnification for sorting of samples.

6.2.1.2 **Compound light microscope**, equipped with a mechanical stage and medium (e.g. 40 ×) and high power (e.g. 100 ×) objectives. The microscope should incorporate facilities for measurements (e.g. an eyepiece graticule) with a resolution of at least 1 µm. Use of a phase contrast or differential interference (Nomarski) condenser may be useful.

6.2.1.3 **Microscope slides and cover glasses**

6.2.1.4 **Immersion oil, dispenser, lens papers and absorbent tissues**

6.2.1.5 **Floras, identification guides and iconographs (illustrations)**, appropriate to the habitats under consideration.

6.2.1.6 **Facility for recording data**, as they are collected. This can be a *pro forma* sheet with a list of taxa and space beside each on which the abundance estimation can be made or a laboratory notebook organised in such a way that taxon identities and abundance can be clearly recorded.

6.2.2 Optional laboratory equipment

6.2.2.1 **Apparatus for photo-microscopy or digital image capture**

6.2.2.2 **Tissue homogenizer or blender**

6.2.2.3 **Magnetic stirrer and stir bar, forceps**

6.2.2.4 **Tally counter**, for species proportional count.

6.2.2.5 **Apparatus and equipment for preparing diatom samples** (see EN 13946)