SLOVENSKI STANDARD
oSIST prEN 15708:2007

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

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 230.

If this draft becomes a European Standard, 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.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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Foreword

This document (prEN 15708:2007) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

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) [2] requires monitoring of the phytobenthos as one part of 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 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 macrophytes are available (EN 14184), this document focuses on phototrophic algae and oxygenic cyanobacteria that live on substrata. However, the focus of this document is shallow running waters which are generally erosive and dominated by boulders, cobbles and pebbles and, under such circumstances, there are often competitive interactions between the larger algae and bryophytes. Similarly, macrophyte species may, themselves, act as substrata 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). Such organisms fall outside the definition of “phytobenthos *sensu stricto*” assemblage.

Methods using phytobenthos to assess water quality in running water have been developed in several European countries and in the USA [1]. Recent work is summarised in the proceedings of three symposia [6], [9], [10]. 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 14184, *Water quality — Guidance standard for the surveying of aquatic macrophytes in running waters*

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

EN 14996, *Water quality — Guidance on assuring the quality of biological and ecological assessments in the aquatic environment*

EN 15204, *Water quality — Guidance standard for the routine analysis of phytoplankton abundance and composition 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

3.2
assemblage
the organisms that share a habitat or microhabitat

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, along which the area covered by phytobenthos is estimated

3.4
benthic algae
algae and oxygenic cyanobacteria living attached to substrata (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
stones with a diameter > 256 mm

3.7
bryophytes
a collective term for liverworts, mosses and hornworts – plants which are often abundant on boulders and bedrock of upland streams

3.8
cobble
stones with a diameter from 64 mm to 256 mm

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

3.10
epilithic algae
algae living attached to or in close association with stony substrata

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

3.12
epipelic algae
algae that live in or on fine sediments

3.13
epipsammic algae
algae that live attached to or in close association with sand

3.14
habitat
the type of environment where individuals of a species live

3.15
macroscopic benthic algae
multicellular algae and aggregations (units/groupings) of unicellular algae living attached to substrata (as opposed to those suspended in the water column) that are 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
a 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 macrophytes, particularly in slow flowing rivers where 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 standard, facultative phototrophs such as many Euglenophyta are included within this definition.

3.19
phytobenthos
all phototrophic algae and oxygenic cyanobacteria that live on substrata, rather than suspended in the water column

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.

3.21
reference conditions
conditions reflecting a totally undisturbed state, lacking human impact, 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

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

3.24
taxon (pl. taxa)
taxonomic unit, such as family, genus or species

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) all microscopic algae found on a single substratum. 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 longer-term storage is necessary, then one or more of the following reagents may be necessary (see 8.4).

5.1 Acid Lugol's iodine

Dissolve 100 g KI (Potassium Iodide) in 1 l of distilled or demineralised water; then add 50 g iodine (crystalline), shake until it is dissolved and add 100 ml of glacial acetic acid. As the solution is near saturation, any possible precipitate should be removed by decanting the solution before use. Lugol's solution can be stored in a dark bottle at room temperature for at least 1 year.

5.2 Alkaline Lugol's iodine

Dissolve 10 g of KI (Potassium Iodide) in 1 l of distilled or demineralised water; then add 50 g iodine (crystalline), shake until it is dissolved and add 100 g NaAc (Sodium Acetate, CH_3COO-Na). As the solution is near saturation, any possible precipitate should be removed by decanting the solution before use.

Lugol's iodine can be stored in a dark bottle at room temperature for at least 1 year.

5.3 Buffered 30 % formalin (formaldehyde)

Formaldehyde (37 %) is diluted to 30 % with distilled water and 100 g hexamethylen-tetramin (hexamin) added per litre. The inclusion of 1 g l^{-1} copper nitrate in a formalin solution helps to maintain the colour of chloroplasts of green algae.

Lugol's iodine and formalin for field use should be stored in small bottles with a tight screw cap and pipette dispenser. The bottle should be kept in a box or solid plastic bag during transport. They should be used in a cup ventilator or fume cupboard in the laboratory.

6 Equipment

6.1 Field equipment

Necessary:

6.1.1 Appropriate water safety equipment

6.1.2 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;

6.1.3 Waders

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

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

6.1.6 Hand lens

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

6.1.8 Sample vials with tight fitting lids

Recommended sizes are 5 ml and 125 ml, to encompass both singular macroscopic units and composite samples.

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

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

6.1.11 Preservative, buffered formalin, Lugol's iodine, or other.

Optional:

6.1.12 Global position satellite (GPS) receiver

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

6.1.14 Bucket, to transfer large substrata to laboratory.

6.1.15 Camera or video-camera

6.1.16 Portable refrigerator or ice box

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

6.2 Laboratory equipment

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

6.2.2 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.

6.2.3 Microscope slides and cover glasses

6.2.4 Immersion oil, dispenser, lens papers and absorbent tissues

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

6.2.6 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.

Optional

6.2.7 Apparatus for photo-microscopy or digital image capture

6.2.8 Tissue homogenizer or blender

6.2.9 Magnetic stirrer and stir bar, forceps

6.2.10 Tally counter, for species proportional count.

7 Survey and sampling strategy

7.1 Approaches

Any reach within a river or stream is likely to contain a number of different substrata on which phytobenthos can grow, and some of these substrata may, in turn, be colonised by more than one type of phytobenthic growth form. In many cases, these growth forms will be visible to the naked eye, even if the constituent organisms are microscopic. In some cases, the growth form will contain one, or a few, dominant organism(s) along with epiphytes and loosely-associated taxa. The dominant organism(s) may be identified in the field, although it may be necessary to confirm the identity in the laboratory. In other cases (e.g. epilithic biofilms), the dominant organism may be too small to be identified in the field. The result is that any survey unit will contain a wide variety of growth forms that may need to be recorded and/or sampled.

Analysis of the phytobenthos at a site / survey unit consists of three stages, which can be combined in various ways to give a number of survey / sampling strategies, each applicable to different purposes. These stages are:

— **Survey:** a detailed inspection of a defined length of the river or stream, recording the nature of the stream environment, the substrata available for phytobenthos and the nature and abundance of any phytobenthic growth forms present.

— **Sampling:** removal of small quantities of some or all the phytobenthic growth forms for subsequent examination in the laboratory.

— **Laboratory analysis:** identification and abundance assessment of the organisms present in the growth forms.

In a few cases (e.g. *Hildenbrandia rivularis*), species-level determinations can be made in the field but in most cases, the identities of macroscopic algae and bryophytes should be checked in the laboratory unless the surveyors have proven competence in field identification of these organisms.

These three stages are combined, in different ways, to give the following sampling strategies:

— **Macroscopic Phytobenthos Survey (MPS):** detailed survey of all, or a selection, of the phytobenthic growth forms that are visible with the naked eye, with sampling and laboratory analysis confined to checking the identities of macroscopic algae and bryophytes. MPS provides semi-quantitative (or, with slight modification, quantitative) estimates of the abundance of those taxa that are visible to the naked eye. It is recommended for trend monitoring and, particularly, for detecting changes in abundance of “nuisance” algae such as *Cladophora* and *Hydrodictyon*.

— **Multi Habitat Sampling (MHS):** survey and sampling of all available habitats / substrata in order to compile a list, with semi-quantitative estimates of abundance, of all phytobenthic taxa present at a survey unit. MHS best characterises the phytobenthos in the reach, but results may not be sensitive to subtle water quality differences because of habitat differences between reaches.

— **Single Habitat Sampling (SHS):** a single type of habitat / substratum is sampled at each survey unit and examined in the laboratory. The output is, as for MHS, a list, with abundance estimates, of all taxa present. SHS should reflect water quality differences between streams more precisely than MHS, provided that the same type of habitat / substratum is sampled at all sites. Impacts on other habitats / substrata in the reach may however be missed. It is identical, in principle, to methods described in EN 13946, except that groups other than the diatoms are included in the subsequent analysis.

Figure 1 is a diagrammatic representation of how these strategies can be applied to a survey unit.