BRITISH STANDARD

BS EN 15708:2009

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

ICS 13.060.70

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British Standards

National foreword

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

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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of an identical text or by endorsement, at the latest by May 2010, and conflicting national standards shall be withdrawn at the latest by May 2010.

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

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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.

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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

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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]

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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.

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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)

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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 optionally 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 those algae such as *Cladophora* and *Hydrodictyon* which are capable of proliferating to "nuisance" quantities.

— **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.

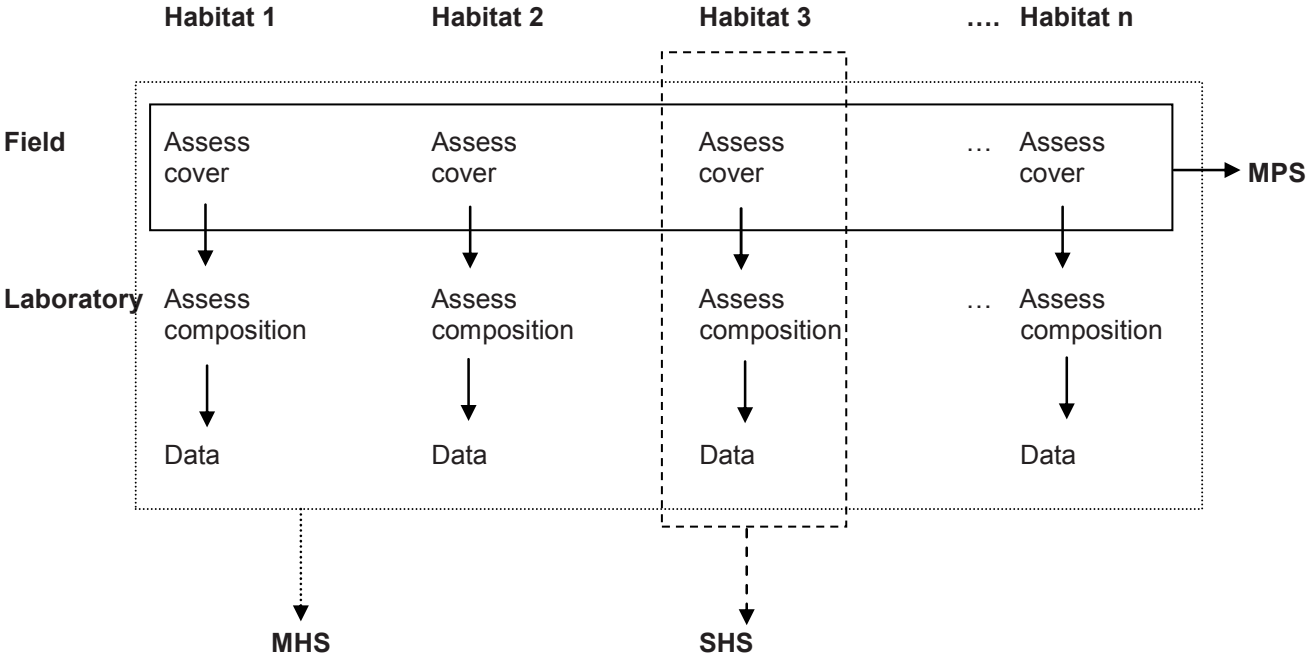

Figure 1 — Relationships between field and laboratory stages for the three types of analysis described in this European Standard

7.2 Number and location of sampling sites

The number and location of sampling or survey sites should be determined according to the aim of the study. In general, sites selected should be representative of the reach of river under consideration, avoiding heavily-shaded sites unless these are characteristic of the reach. Where possible, sites selected should have similar conditions of light, current velocity, substratum, etc. to facilitate comparability.

Reaches should be identified within a watercourse with respect to points where marked changes in quality are likely to occur or where there are important river uses, for example major discharges or abstractions. Sufficient sample sites should be included within each reach to differentiate naturally occurring changes (geology, climate, etc.) from changes caused by human impact. If sampling is intended to monitor the effects of a discharge, sample sites upstream and downstream (beyond the mixing zone) of the discharge should be included. Additional sites should be added at distances downstream to assess the extent of the influence of the impact, and any possible recovery.

The location of the selected survey unit within a given site should be defined in field notes by reference to map co-ordinates and location relative to permanent bankside objects (e.g. bridges). GPS (global positioning system) receivers may be useful for obtaining accurate co-ordinates. It may also be appropriate to indicate the location of a site by the use of permanent markers such as iron bolts or paint on large stones.

The survey units should all be the same length: 10 m is recommended, but longer lengths may be appropriate under some circumstances. The samples should be collected from the main channel of the river (i.e. the zone that is normally submerged). Rivers with naturally fluctuating discharge have specific algal associations in the flood zone that may be of interest to sample.

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7.3 Time of sampling

The time of sampling depends on the scope of the survey and the local conditions. Seasonal runoff patterns should be considered. It is recommended that surveys are performed and samples collected during periods of stable water flow, preferably at low discharge conditions.

The composition of stream phytobenthos varies throughout the year. A single sample or survey may not be sufficient to characterise fully the diversity in a reach. One sample or survey may, however, be sufficient for spatial and temporal comparisons, as long as all the samples are collected, or surveys performed, at the same time of year.

8 Survey and sampling procedures

8.1 Field survey method: MPS - Macroscopic phytobenthos survey

A detailed examination of the entire survey unit should be performed by wading, using an aquascope if observation of the stream bed is hampered by depth or surface turbulence. If some parts are too deep for safe wading, then the survey should cover all areas that can be waded, and a note made. The length of the survey unit shall be recorded. The presence of all phytobenthic growth forms shall be recorded and their abundance estimated. Small specimens should be removed for either bank-side or laboratory examination. The macroscopic growth forms visible with the naked eye may have different appearances, e.g. gelatinous brown cover (often diatoms), green filaments (usually green algae) or dark tufts (red algae or cyanobacteria). The largest filamentous algae may be > 2 m long, whilst some other growth forms may be only 1 mm or 2 mm (e.g. *Heribaudiella, Chamaesiphon* spp.), and only just visible to the naked eye. Great care shall be taken during this survey as the smallest algal growth forms may be only 1 mm or 2 mm across and easily missed. A checklist of growth forms that may be encountered should be prepared in advance of the survey. If growth forms cannot be classified into one of these classes, then the nature of the growth form should be described (size, colour, appearance) separately on the field record sheet. Additional notes (e.g. recording the size and colour) should also be taken, if appropriate. The abundance estimates should only represent the length of river surveyed (survey unit). The length of the survey unit shall be recorded.

NOTE 1 It can be useful to record the identities and abundance of aquatic bryophytes and vascular plants present in the survey unit.

NOTE 2 In some cases (e.g. when monitoring algae capable of causing nuisances), it can only be necessary to record and assess the abundance of one or a few species or growth forms.

Samples of those taxa and growth forms that cannot be identified *in situ* should be collected and examined in a tray (6.1.1.7) filled with some streamwater to get an impression of colour, texture, etc. A hand lens (6.1.1.6) may be useful. Details of sampling procedures are given in 8.2. Those taxa that cannot be identified in the field should be taken back to the laboratory for confirmation. Small quantities of macroscopic algae (and optionally bryophytes) should be retained as voucher specimens.

The abundance of each macroscopic growth form should be estimated using a simple descriptor scale, based on the percentage of the stream or river bed (within the survey unit) that is covered (Table 1). The quantification is based on “qualified judgement”. Scales with about five levels balance the needs for reproducibility and spatial/temporal discrimination. Scales with a greater number of levels may appear to be more accurate but may be less reproducible.

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Table 1 — Descriptor-scale for abundance estimates of macroscopically visible growth forms

Scale	Description of coverage at the survey unit
1	*Rare*: just visible in the field, covers < 1 % of the river bed
2	*Occasional*: covers 1 % to < 5 % of the river bed
3	*Frequent*: covers 5 % to < 25 % of the river bed
4	*Abundant*: covers 25 % to < 50 % of the river bed
5	*Dominant*: covers ≥ 50 % of the river bed

Depending upon the survey aims, it might be appropriate to use a more precise estimation of abundance. Options include:

— a nine-point scale for estimating biomass in the field, based on percent cover of macroalgae and the thickness of these growths, is given in [8];

— percent cover along one or more "belt transects";

— percent cover in a number of random squares;

— percent cover according to specified grid/quadrats.

Details of these methods are beyond the scope of this European Standard.

8.2 Sampling method: MHS - Multi habitat sampling

Survey growth forms present as for MPS. Collect at least three sub-samples of approximately equal size from each growth form identified during the MPS following methods in Table 2 and place these in a single container to give one composite sample per growth form. A knife, blade, forceps or stiff brush is used to collect samples. If there are obvious differences in appearance of a growth form on different substrata then sub-samples should be collected from each substratum and stored separately. Pick specimens of macroalgae and bryophytes by hand and place each in a separate container. In order to keep the material as fresh as possible, sample bottles should contain no more than 10 % to 20 % of algal material plus stream water, in order to ensure that there is sufficient air for gas exchange to take place.

Note that the composition of some growth forms may vary depending upon the stability of the substratum. For this reason, separate samples may need to be collected from large (cobbles, boulders) and small (pebbles, gravel) substrata. Sand and silt may also each have their own distinctive floras.

Encrusting algae (such as *Gongrosira incrustans*) often need to be examined under a microscope in order to confirm identities. Cobbles containing these should be placed in plastic bags or in a bucket, covered with stream water, for return to the laboratory.

Portions of macrophyte with visible algae should be placed, along with a little stream water, either in a bottle or a plastic bag for transport to the laboratory.

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Table 2 — Collection techniques for phytobenthos in running waters used in MHS

Habitat/Substratum Type	Collection Technique
Removable substrata (hard): gravel, pebbles, cobble	Remove representative substrata from water; brush or scrape representative area of phytobenthos from surface and rinse into sample vial. NOTE This procedure is identical to that described in EN 13946 for benthic diatoms.
Removable substrata (soft): bryophytes, macroalgae, vascular plants (decaying organic material/objects should not be sampled)	Place portion of the plant in a sample container with some water. Shake it vigorously and rub it gently to remove phytobenthos. Remove plant from sample vial. Pieces of macrophytes, etc. with visible algae should be collected and stored in a separate bottle/plastic bag.
Large substrata (not removable): boulders, bedrock, logs, trees	Place PVC pipe (diameter 10 cm to 15 cm) with a plastic collar at one end of the substratum so that the collar is sealed against the substratum. Dislodge phytobenthos in the pipe with a knife, blade, forceps or stiff brush. Remove phytobenthos from pipe with pipette.
Loose sediments: sand, silt, other fine particulate matter[a]	Invert Petri dish over sediments. Trap sediments in Petri dish by inserting spatula under dish. Remove Petri dish from the river whilst holding the spatula in place under the dish, and rinse into sampling container. Samples from depositional habitats can also be collected with spoons, forceps, capillary tube or pipettes.

[a] Loose sediments seldom contain large amounts of algae and may be omitted from sampling unless algal growth is obvious. Loose sediments should, if sampled, be kept in separate vials. Specialist laboratory treatment is required to separate algae from the inorganic matrix.

8.3 Sampling method: SHS – Single habitat sampling

This is a variant of 8.2 in which the same type of substratum is sampled at all sites included in a survey. Ideally a single hard and inert substratum should be sampled. The recommended substratum is cobbles obtained from riffles and runs with current velocities of 20 cm/s to 50 cm/s. Sampling from riffles ensures a continuous exchange of the water and prevents the build up of a local chemical environment. Further, it prevents sedimentation of drifting organisms and fine particulate matter.

At least five cobbles of similar size should be removed, placed in a tray with a little stream water and the phytobenthic growth forms removed by brushing with a toothbrush. The resulting suspension should be placed in a single container. The percentage of the total area covered by this substratum should be recorded on the field record sheet. Pick specimens of macroscopic growth forms by hand and place each in a separate container.

For some river types, cobbles may not be the most appropriate substratum to sample. Alternative substrata include macrophytes and other hard surfaces, as long as these have been in the river for at least four weeks prior to sampling. Decaying wood and other dead organic substrata should not be sampled. The selected substratum/habitat and the number of sub-samples should be specified in the field record.

8.4 Preservation

If treated with care, many algal samples can be stored in the dark in a refrigerator or cool room for a few days without deterioration (maximum 48 h if they are from a polluted location, and never more than five days). This is the recommended approach; however, if this is not possible, one of the preservatives described in Clause 5 should be added or the sample should be frozen. Some types of macroalgae can also be dried. No single preservative can be recommended for all circumstances and the following points should be borne in mind when selecting a preservation method:

— Acid Lugol's iodine may, if too concentrated, dissolve the frustules of diatoms. It is also unsuitable for use with calcareous algae;

— Acid Lugol's iodine prevents the loss of flagella, so is useful if flagellated algae are likely to be common in samples;

— Lugol's iodine is absorbed by inorganic sediments. The extent to which this occurs will vary, depending upon the type and quantity of sediment, with the result that over or under-fixation is common;

— Lugol's iodine destroys the gas vacuoles of Cyanobacteria, making these harder to identify;

— Lugol's iodine stains the starch grains of Chlorophyta and can make these easier to distinguish from representatives of Xanthophyta which do not have starch as their storage product;

— Lugol's iodine sublimes from most types of container over time, so should not be used for long-term storage;

— Freezing can disrupt the structures of some of the more delicate organisms;

— All preservatives have associated health risks and should only be used in accordance with appropriate health and safety precautions.

In some cases, particularly where detailed taxonomic studies are required, a pair of samples, each preserved with a different preservative, may be required.

One to five drops of Lugol's iodine should be added per 100 ml sample to give a final "straw" colour. More may be necessary if samples are rich in organic matter. Neutral formalin may be used to preserve samples that are stored before analysis. To a sample vial that contains 10 ml liquid is added five to six drops 30 % formalin (one drop formalin equals approximately 30 µl). This gives 0,5 % to 0,6 % formalin in the final sample, which is sufficient as long as the sample does not contain material from a severely polluted location, in which case two to four drops extra should be added. 10 % formalin can also be used, adjusting the amount added to give an equivalent final concentration.

For long term storage (one year or more) add ten drops of 30 % formalin to 10 ml of liquid (equals 1 %). A pair of samples, each preserved with a different preservative, may be required, depending upon the composition of the sample.

CAUTION — The use of formalin can cause health problems.

NOTE 1 For more details on the use of preservatives see EN 15204:2006, Annex B and [4].

Bryophytes and higher plants should be transferred to the laboratory in a moist condition (e.g. in a plastic bag or sample bottle) in order to preserve any epiphytes in a healthy state. Once the epiphytes have been examined, voucher specimens may be prepared by pressing and drying the plant material using blotting paper.

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8.5 Labelling of sample containers

Sample containers (plastic bags, vials, bottles) should be labelled with pencil or indelible marker on waterproof paper that is put inside the sample container, and outside if desired. The following information should be given:

— name of watercourse, survey unit identification, preferably as a specified code;

— sampling approach (MPS, MHS, SHS);

— samples collected should be marked consecutively A, B, C or the like;

— date of sampling, name of collector.

8.6 Recording data in the field

A field-recording sheet should be filled out for each sampling survey unit. This can be a standardised recording sheet (pro forma) or a notebook with waterproof pages.

A detailed description of the site/survey unit is required on the first occasion that it is surveyed/sampled. Information that should be recorded is:

— locality identification; including name of watercourse, locality name, locality code. The position of the survey unit/sampling points shall be defined unambiguously, such that other operators can accurately relocate them. Positions should be defined using geographic co-ordinates with reference to the relevant system for graticules (such as European Datum: ED-50, World Geographic System: WGS-84), and also using the UTM system, if appropriate. Positions should be defined according to the relevant guidelines;

— description of upstream and downstream limits of the survey unit (permanent markers/bankside objects);

— date;

— name/initials of the person sampling;

— sampling approach (MPS, MHS, SHS).

Record according to sampling approach:

— *MPS-sampling*. Description of each macroscopic growth form, estimate of its abundance and corresponding sample identifier (A, B, C, etc.);

— *MHS-sampling*. Number of sub-samples in a composite sample; description of each macroscopic growth form and corresponding substratum/habitat, estimate of its abundance and corresponding sample identifier (A, B, C, etc.);

— *SHS-sampling*. Substratum/habitat type, number of sub-samples in composite sample, estimate of its abundance and corresponding sample identifier.

Other information that should be recorded in the field records depends on the type of survey and the type of locality. After sampling, review the recorded information on all labels and field sheets/book for accuracy and completeness.

8.7 Storage of samples and field records

Log all incoming samples as soon as they arrive at the laboratory. As a minimum, record sample identification code, name of watercourse, sampling location, date and collector's name. Samples should be stored in a cool, dark place until they are examined. Samples should be preserved shortly after they are examined. This should be done as described in 8.4. Storage in an ordinary room with good ventilation is acceptable (for preserved material). Samples should be stored at least one year after they have been examined to enable independent verification of composition, if required.

9 Identification and basic quantification of organisms

9.1 Preparation of the microscope

The eyepiece graticule, or other measuring equipment should be calibrated against a stage micrometer. The results of this calibration should be displayed in a position where users of the microscope can consult them. A resolution of 1 µm is adequate for routine analyses. The second eyepiece may be equipped with a second graticule to aid abundance estimation/enumeration.

9.2 Analytical procedure

Representative sub-samples should be taken from the sample(s) collected from each habitat/substratum. If the material is not homogenous, then it may be necessary to macerate the sample first, using either dissecting instruments or a tissue homogeniser.

Use a Pasteur pipette to place a small quantity of the sub-sample onto a microscope slide and place a coverslip on top. If necessary, add one or two drops of water.

Examine the sample under medium power objectives (approx. 400 × magnification) and identify and record all phototrophic organisms present. In the case of diatoms, base abundance estimates on those cells that contain healthy chloroplasts only. Repeat this for at least three replicate sub-samples per habitat/substratum then make an estimate of the abundance of each taxon on a semi-quantitiative scale. A five level descriptor scale is recommended (Table 3). In some cases, high power (oil immersion) objectives should be used to confirm identities.

It is possible to use counting chambers to enumerate algae. Reference [6] gives information on the use of a Palmer chamber on a conventional microscope whilst the Utermöhl method, using an inverted microscope (described in EN 15204) can also be used. These methods are however beyond the scope of this standard.

Table 3 — Descriptor scale for abundance estimates of microscopic growth forms

Scale	Description
1	*Rare* – one or very few cells or coenobia, or short filaments are observed.
2	*Occasional* – the taxon is seen several times during scans of wet mounts of a particular sample, but never in great numbers.
3	*Frequent* – a representative of this taxon is present in most fields of view observed during scans of wet mounts.
4	*Abundant* – more than one cell or coenobium of the taxon is present in most fields of view observed during scans of wet mounts.
5	*Dominant* –the most common organism(s) observed in all scans of all sub-samples collected from a particular habitat.

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Most macroscopic plants will have epiphytes. Wet mounts should be made of small portions of filaments and bryophyte leaves so that they can be examined directly under the microscope. Leaves of higher plants can be scraped with a blade or a needle, and the epiphytic flora examined in the same way.

The appropriate taxonomic identification level will depend upon the aims of the study and should be decided in advance. It may also vary from one taxonomic group to another. Some specimens are easy to identify to species level, e.g. the chrysophyte *Hydrurus foetidus* and this is recommended wherever possible. Others can only be identified to generic level, unless they are cultured to produce reproductive bodies, e.g. the filamentous green alga *Mougeotia*. Some are even difficult to identify to generic level, e.g. some coccoid cyanobacteria. In the case of filamentous algae that lack reproductive structures, the width of the filament should be recorded in order to distinguish between different forms even when species-level identification is not possible.

If diatoms are abundant in samples, methods for cleaning diatom samples are described in EN 13946; and methods for enumeration and identification of diatoms are described in EN 14407.

10 Data processing and interpretation

10.1 Final abundance estimate

In the case of MPS the outcome will be a list of taxa along with 5-point semi-quantitative estimates of abundance that can be processed directly (Table 1). However, in the case of MHS and SHS, the outcome will be two lists of data: field estimates of the abundance of each phytobenthic growth form (Table 1) plus estimates based on laboratory analyses of the abundance of each constituent organism in the growth form (Table 3), each on a 5-point scale. These can be combined to produce a single, integrated estimate of the abundance of each taxon at the survey unit (Tables 4 and 5). For microscopic constituents of an assemblage, both the abundance estimate for the assemblage/growth form in the field (A in Table 4) and the abundance estimate for the taxon in question obtained in the laboratory (B in Table 4) are combined to produce the integrated estimate (C in Table 4). If the same microscopic species is found in several of the assemblages, then the final value for these species should be the highest of those recorded in the assemblages.

Annex A shows a worked example, based on a stream flowing off blanket bog in NE England.

For macrosocopic constituents (e.g. macroalgae), the abundance estimate in the field should be quoted directly (as in MPS).

Table 4 — Final abundance estimates for MHS method, based on separate 5-point scales for field survey (Table 1) and laboratory analysis (Table 3)

A: Abundance in field, see Table 1	**B: Abundance in laboratory, see Table 3**				
	1 Rare	2 Occasional	3 Frequent	4 Abundant	5 Dominanrt
	C: Integrated estimate of abundance				
1 (< 1 %)	1	1	1	1	1
2 (1 % to < 5 %)	1	2	2	2	2
3 (5 % to < 25 %)	1	2	3	3	3
4 (25 % to < 50 %)	1	2	3	4	4
5 (≥ 50 %)	1	2	3	4	5

Table 5 — Final 5-point scale for estimating abundance of phytobenthos in streams that combines the 5-point field scale and the 5-point laboratory scale, see Table 4

5-point scale value	Final abundance estimate	Description
1	Rare	Macroscopic organisms with field value 1 (cover < 1 % of stream bed) - or microscopic organisms found in phytobenthic growth forms with field value 1 - or which are found rarely (lab. value 1) during laboratory analysis of growth forms with higher field values (2 to 5).
2	Occasional	Macroscopic organisms with field value 2 (cover 1 % to ≤ 5 % of stream bed) - or microscopic organisms which are at least occasional (lab. value 2 to 5) in growth forms with field value 2 - or which are found occasionally (lab. value 2) in growth forms with higher field values (3 to 5).
3	Frequent	Macroscopic organisms with field value 3 (cover 5 to < 25 % of stream bed) - or microscopic organisms which are at least frequent (lab. value 3 to 5) in growth forms with field value 3 - or which are found frequently (lab. value 3) in growth forms with higher field values (4 to 5).
4	Abundant	Macroscopic organisms with field value 4 (cover 25 % to < 50 % of stream bed) - or microscopic organisms which are at least abundant (lab. value 4 to 5) in growth forms with field value 4 - or which are abundant (lab. value 4) in growth forms with higher field value (5).
5	Dominant	Macroscopic organisms with field value 5 (cover ≥ 50 % of the stream bed) - or the dominant microscopic organism(s) (lab. value 5) in growth forms with field value 5.

10.2 Interpretation of data

Research into the use of phytobenthos to evaluate ecological status is underway in several European countries. Recent summaries of such work are given in references [1], [7], [11] and [12]. Few indices have been developed specifically to use the entire phytobenthos (rather than just diatoms) for monitoring water quality. Two that are available are the Saprobic index for organic pollution [8] and Trophic index for nutrient pollution [9]. The Saprobic index has variants suitable for use with macroalgae, all microscopic algae and diatoms alone. These were developed for use in a single geographical area although subsequent testing has shown several to have broader validity. The composition of the phytobenthos also indicates other pressures (e.g. acidification, toxic substances, nutrients [6]).

11 Quality assurance

The validity of ecological assessments based on phytobenthos depends on the accuracy and precision of all activities involved in the collection and analysis of data. For this reason, assessments should be subject to quality assurance procedures. For more details, reference should be made to EN 14996.

Annex A
(informative)

Worked example of computation of final abundance estimates for a MHS from an upland stream in NE England

Table A.1 — Worked example of computation of final abundance estimates for a MHS from an upland stream in NE England

Taxon	Field cover	Lab estimate	Final abundance estimate	
***Scapania undulata* epiphytes**				
Eunotia exigua	3	1	1	
Gomphonema sp.	3	1	1	
Microspora pachyderma	3	1	1	*
Green floc (1)				
Chamaesiphon incrustans	3	2	2	
Closterium leibleinii	3	2	2	
Cosmarium subcrenatum	3	1	1	
Cryptomonas erosa	3	2	2	
Cylindrocystis sp.	3	1	1	
Eunotia exigua	3	1	1	*
Klebsormidium rivulare	3	1	1	
Microspora pachyderma	3	4	3	
Mougeotia > 8 µm to 12 µm	3	4	3	
Navicula slesvicensis	3	1	1	
Pinnularia appendiculata	3	1	1	
Staurastrum punctulatum	3	1	1	
Synura petersenii	3	1	1	
Tabellaria flocculosa	3	1	1	
Zygnema >8 µm to 12 µm	3	1	1	
Green filaments				
Microspora pachyderma	3	5	3	*
Euglena mutabilis	3	1	1	
Pinnularia appendiculata	3	1	1	*
Closterium leibleinii	3	1	1	*
Green floc (2)				
Mougeotia > 8 µm to 12 µm	2	5	2	*
Closterium leibleinii	2	1	1	*
Cylindrocystis sp.	2	1	1	*
Phormidium > 8 µm to 16 µm	2	1	1	
Cosmarium subcrenatum	2	1	1	*
Klebsormidium rivulare	2	1	1	*
Eunotia exigua	2	1	1	*

Explanation:

Four assemblages (see 3.2) were identified during the field survey.

Asterisks indicate taxa that were found in another assemblage at the same or a greater final abundance estimate.

Some filamentous taxa are recorded as width categories as reproductive organs were not present (see 9.2).

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