BS EN 14407:2014

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Water quality — Guidance for the identification and enumeration of benthic diatom samples from rivers and lakes

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National foreword

This British Standard is the UK implementation of EN 14407:2014. It supersedes BS EN 14407:2004/BS 6068-5.35:2004 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee EH/3/5, Biological Methods.

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

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

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

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Foreword

This document (EN 14407:2014) 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of an identical text or by endorsement, at the latest by September 2014 and conflicting national standards shall be withdrawn at the latest by September 2014.

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.

This document supersedes EN 14407:2004.

This document contains the following technical changes compared with the previous edition:

— This European Standard is now also applicable for the identification, enumeration and interpretation of benthic diatoms in lakes, i.e. not only rivers.

— Informative Annex A “Example for quality assurance of diatom analyses from the UK” was added.

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

Introduction

Diatoms are an important component of aquatic ecosystems and constitute a water quality monitoring tool where the primary objective is either a measure of ecological status or the impact of specific components of water quality (e.g. eutrophication, acidification). The requirement for the monitoring of such processes is inherent in the Water Framework Directive (2000/60/EC) and Urban Waste Water Treatment Directive (91/217/EEC) in addition to other EU Directives and international agreements. This European 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 river and lake quality is widely accepted both in Europe and the USA. The methodology is based on the fact that all diatom species have tolerance limits and optima with respect to their preference for environmental conditions such as nutrients, organic pollution and acidity. Polluted waters will tend to support an increased abundance of those species whose optima correspond with the levels of the pollutant in question. Conversely, certain species are intolerant of elevated levels of one or more pollutants, whilst others can occur in a wide range of water qualities.

Methods using diatoms to assess water quality have been developed in several European countries (recent work is summarized in the proceedings of three symposia [4] to [6]. The methodologies for evaluating the diatom data vary but the sampling and preparation processes are similar [1].

According to the precise usage to which this European 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.

All numerical values given in this standard are approximate.

WARNING — Persons using this European Standard should be familiar with usual laboratory practice. This European 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.

1 Scope

This European Standard specifies 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 lakes. It is suitable for use with indices and assessment methods based on the relative abundance of taxa. The methods for identification and enumeration may also be applied to the study of benthic diatoms in other habitats provided that data interpretation methods appropriate to these habitats are used.

2 Terms and definitions

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

2.1
benthic diatoms
diatoms living on natural or artificial substrata, rather than suspended in the water column

2.2
ecological status
measure of the structure and functioning of aquatic communities

2.3
expected natural assemblage
assemblage present at a site when only natural stresses (e.g. floods) occur and man-made stress is not significant

2.4
eyepiece graticule
measuring device, inserted into one eyepiece of a microscope, permitting measurements of the size of objects

Note 1 to entry: The relationship between each division on the eyepiece graticule and the actual size of the object will depend upon the magnification of the microscope.

2.5
frustule
cell wall of diatoms, composed of silica and consisting of two valves linked by two or more girdle bands

2.6
habitat
specific environment in which an organism lives

2.7
prepared slide
slide plus coverslip on which has been mounted a sub-sample of diatoms

2.8
stage micrometer
special graticule in the form of a scale carried at natural size on a microscope slide which is used as an absolute standard of length for calibrating microscope measuring systems

[SOURCE: ISO 10934-1:2002, 2.96.1]

2.9
taxon (pl. taxa)
taxonomic units, for example families, genera or species

2.10
unit
either diatom valves or intact frustules

Note 1 to entry: The use of the term is depending upon the conventions adopted in 5.2.

2.11
valve
structural component of the diatom frustule

2.12
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 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.

4 Apparatus

4.1 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 scale division 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.

4.2 Floras, identification guides and iconographs, appropriate to the habitats under consideration (see 5.1).

4.3 Immersion oil and dispenser

4.4 Lens tissue

4.5 Facility for recording data as they are collected.

This may 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 organized in such a way that taxon identities and numbers can be clearly recorded, or a computer program with facilities for direct entry of data.

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

4.6 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 coordinates 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.

5 Determining analytical strategy

5.1 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 along with the names of taxonomic reference works consulted should be cited in all cases where there is any potential for nomenclatural confusion.

5.2 Determining units for enumeration

Different conventions have evolved for enumeration of diatoms, either using valves or frustules as the basic unit, or not distinguishing between valves and frustules. It is important that the convention is specified and documented in advance.

Because of the distinct definition, the use of valves or frustules (two valves are the equivalent of one frustule) as basic units guarantees the universal comparability between studies and enables further analysis, e.g. the calculation of biomass considering reference data.

In the case of small diatoms, such as e.g. some *Achnanthes* and naviculoid species, it may not be possible to distinguish between intact frustules and isolated valves with certainty on all occasions. The effects of this uncertainty on the enumeration, the relative proportions of diatom taxa and further analysis is likely to be small.

If other definitions which do not distinguishing between valves and frustules (e.g. “objects”), are used as basic units it is essential to define the treatment of colonies and only partly separated frustules precisely. The comparability of such method specific enumeration techniques to other studies may be limited.

5.3 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 units 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. Details of studies with a range of objectives may be found in references [4] to [7].

5.4 Determining approach to enumeration

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 1 µm is adequate for routine analyses. Imaging software connected to video capture equipment may also be used.

The second eyepiece may be equipped with a separate graticule to aid enumeration. This can take several forms: including a square grid, H-shape, Whipple field, etc. Options for enumeration are:

a) 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

b) 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.

c) When the total number of diatom units required is defined rigorously, then a combination of these approaches may be considered, starting with counting within a field of view until close to the target and then finishing with counting along a traverse.

In all cases, the procedure is repeated until the total has been reached.

A further “house rule” is needed to cover situations where a diatom is only partially inside a defined counting area. For example, such a rule might include diatoms that are only partially visible at the upper but not the lower margin (in the case of horizontal traverses) or the left but not the right margin (in the case of vertical traverses). The precise form of this rule is less important than consistency in its use when analysing samples.

Whether a horizontal or vertical traverse is used, it is important that the field of view visible during each subsequent traverse does not overlap with that visible during the previous traverse. The distance that the stage is moved on each occasion should also account for any diatoms only partially visible in a field of view. If sample analysis is unlikely to be completed in a single session, then it is useful to record the position of each traverse using the Vernier scale. This ensures that subsequent traverses do not overlap with those already completed.

NOTE Additional precautions are required if more than one microscope is likely to be used for analysis, as positions on Vernier scales may differ between microscopes.

5.5 Treatment of broken and other unidentifiable diatoms

In order to eliminate the risk of including separate fragments of broken valves or frustules, a consistent approach should be decided before the start of a project. Possible approaches are:

— include a broken individual only if approximately three-quarters is present;

— include a broken individual only if at least one pole and the central area are present;

— exclude all broken individuals.

NOTE The second situation is difficult to apply to taxa that lack an obvious central area. Under these circumstances, the number of individuals present can be assumed to be the total number of poles recorded, divided by two.

A diatom may not be identifiable for a number of reasons, including presentation of a girdle view, the presence of overlying material obscuring a clear view or the taxon not being recognized by the analyst. If overlying material obscures many valves, then new slides should be prepared using more dilute suspensions or by adjusting settling times to separate diatoms from contaminants.

Some taxa are identifiable from girdle views, either because the girdle view is particularly characteristic (e.g. *Rhoicosphenia abbreviata*) or because the girdle view can be assigned with confidence to a particular taxon by “matching” it with corresponding valve views of taxa found in the sample. However, this is not always possible and, if in doubt, the analyst should record the girdle views at the lowest level to which they can be assigned with confidence (e.g. “unidentified *Gomphonema* sp.”, “unidentified pennate girdle view”).

This convention should also be applied to any other individuals found on the slide but not identifiable by the analyst. A large number of such individuals may indicate a problem with either slide preparation or the identification skills of the analyst. The approach that should be adopted will depend upon the assessment method proposed. Not all taxa need to be identified for some indices to be used. However, for indices, where

there is a presumption that all taxa in a sample are identified, it is recommended that not more than twelve percent of the total count should be composed of individuals that have been not identified to the species level. For indices, based on genera or a mix of species and genera, not more than five percent of the total count should be composed of individuals that have not been identified at least to genus.

6 Analytical procedures

The term “diatom unit” is used in this section to avoid problems outlined in 5.2. This term can refer to either valves or frustules, depending upon the conventions adopted for a particular study.

a) Place a slide on the stage and copy relevant information from the slide label to the record sheet or computer program. The recommended minimum information is sample number, water body name, site name, and sample date. Other essential data are the date of the analysis and analyst’s name.

b) Select an appropriate starting position on the slide. The edge of the dried sample suspension is recommended, but if this is adopted, ensure that there are no significant “edge effects” (i.e. no taxa are obviously more concentrated at the edge, compared to elsewhere, on the dried suspension).

If the distribution is not homogeneous, a new slide should be prepared.

An alternative approach to the use of traverses is to use random fields. If this approach is adopted, then random fields should be located using the Vernier scales on the microscope in conjunction with either tables of random numbers or random number functions within computer programs or electronic calculators.

c) Using a high power objective, identify all units present in the first field of view. Use the fine focus mechanism to differentiate between a single valve and an intact frustule, if this distinction is being made. If the basic unit of enumeration is the valve, record an intact frustule as two units.

An intact frustule will have two distinct planes of focus when the striae, raphe and other structures will be clearly visible. Careful use of the fine focus mechanism should enable these to be differentiated. An intact frustule also often has different optical properties to a single valve.

d) Occasional filaments and colonies should be recorded as the corresponding number of diatom units. If large numbers of diatom units are found in filaments and colonies, a new preparation, using a more aggressive mix of oxidising agents, should be considered.

e) If a diatom unit cannot be identified for any reason, follow the conventions outlined in 5.4. Photographs, “captured” digital images or detailed drawings should be made and sent to more experienced colleagues. Notes should also be taken of shape and dimensions of the diatom unit, striae density and arrangement (at centre and poles), shape and size of central area, number and position of punctae and arrangement of raphe endings.

f) Once all taxa within the first field of view have been recorded, the count should continue, following one of the methods in 5.4, and all diatom units identified and enumerated on the data sheet or program. This process should be continued until the required number of diatom units has been counted.

g) For some purposes, it is useful to continue to scan the slide after the required number of diatom units has been counted, and any taxa encountered that were not included in the count should be identified and recorded as “present”. A further scan using a medium power magnification (e.g. 400 ×) may also be appropriate in order to note any larger taxa (e.g. *Gyrosigma, Didymosphenia*), which can escape detection at higher magnifications.

h) At the end of the analysis, the slide should be removed from the mechanical stage and excess immersion oil wiped from the objective and slide.

For example, if *Cocconeis placentula* constitutes 200 units out of a total of 300, then a stratified procedure may be necessary by continuing to count taxa other than *C. placentula* until the total of these is 300. The

number of *C. placentula* then needs to be scaled up by a factor of 3 in order to reflect its relative abundance in the sample. Such an approach allows the enumeration of statistically-significant numbers of sub-dominant taxa; however, the desirability of this approach will depend upon the assessment method used and situations when it should be applied should be specified in detailed guidelines.

7 Archiving data, slides and samples

Diatom slides represent a permanent record of conditions at a site that can be reanalysed in different ways in the future. For this reason, it is important that slides are stored in an appropriate manner, such as lodgement in a local or national herbarium. If this is the case, then slide labels should contain sufficient information to link them in an unambiguous manner to other site details, such as map coordinates, chemical and hydrological data, etc.

The suspension of cleaned diatoms should also be labelled in an unambiguous manner and stored in order to allow further slides to be prepared, if necessary, in the future. Use of a preservative such as ethanol or formalin is recommended in order to prevent microbial growth or chemical dissolution of the diatoms.

Preservation of a representative selection of fixed samples is also recommended, as a means of checking anomalous results.

8 Quality assurance

The validity of ecological assessments based on benthic diatoms 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. See EN 14996 and Annex A, which give examples for such a procedure.

9 Data interpretation

9.1 Use of indices and scores

In order to measure the level of a particular stress, a biological index or score specifically designed to evaluate that stress should be used. This index or score should be based on the relative abundance of taxa present in a sample. The stresses most widely evaluated using benthic diatom assemblages are organic pollution, eutrophication, salinity and acidification. In addition, some indices measure “general water quality”.

References [4] to [6] describe and evaluate many indices used at present. Most of these were developed for use in a single geographical area although subsequent testing has shown several to have broader validity. Before an index is used for the first time in an area, some preliminary evaluation of the index will be necessary. This evaluation should consider the autecological information on the taxa, as well as the physical and chemical conditions at the particular site, in order to provide a sound ecological underpinning to the work. It is important that the dominant and sub-dominant taxa present in the region are represented in the index.

Not all environmental stressors are covered by these indices. For example, high concentrations of heavy metals exert an influence on diatom assemblages that is not readily quantified. If stressors other than those mentioned above are suspected then specialist literature or an expert in the field should be consulted.

9.2 Comparison with reference conditions

In order to evaluate overall levels of environmental stress at a site, the data from the site (called the observed data) should be compared with a set of reference data. The reference data represent the expected natural assemblage that would be found at the site, when only natural stresses are present and man-made stresses can be considered to be insignificant. A classification of sites is based on the disparity between observed data and reference data. Reference [7] summarizes approaches used in Europe for the assessment of “ecological status” using diatoms.

Annex A
(informative)

Example for quality assurance of diatom analyses from the UK

Sampling and analysis of benthic diatoms for ecological assessments should take place within a quality management programme, in order to ensure that outcomes are of a known and verifiable quality.

There have not been enough studies on the variability of benthic diatom samples for a recommendation for the quality management of benthic diatom samples to be included in the normative part of this European Standard. Instead, an account of one national quality management programme has been included in this annex.

An important principle behind this scheme is that there is a human dimension to intercalibration of ecological methods and this scheme exists not just to establish values for process errors, but also as a means of on-going training for participants. The aim is encourage “reflective practice” in all the analysts involved in the scheme.

Uncertainty due to sampling is not considered in this scheme. Instead, global estimates of sampling error are included when evaluating confidence of class and risk of misclassification in the tool itself.

Analysts involved in the scheme are expected to fulfil the following requirements:

— either undergo a training course or demonstrate equivalent prior learning, and then to undergo a competency test in order to become an accredited analyst;

— analyse at least 30 samples per year, in order to maintain their familiarity with the UK’s diatom flora;

— attend at least two days of professional development (scientific meetings, training courses) with a focus on diatoms and freshwater algae;

— participate in the national ring-test scheme; and

— take part in an internal audit scheme.

The ring-test scheme itself works as follows:

a) All those taking part in the scheme are sent five slides during the course of the year, representative of any habitats that they are likely to encounter during routine monitoring. These slides are sent at intervals through the year.

   Participants know which slides are part of the intercalibration scheme. An exercise in which participants cannot distinguish audit from routine slides would have been difficult to put into practice. Participants may, therefore, spend longer on this slide than they would on a routine slide but this extra time is regarding as ‘learning’ which will benefit future analyses.

   They are expected to perform a normal analysis on this slide within a month of receiving it and to send the result to the organizer.

b) At the same time as they are doing this, an ‘expert panel’ of experienced diatomists also analyse the slide. The mean of their results provides the “assigned value” for the exercise. The standard deviation of their results provides an indication of the variability that we should expect in the ring-test results.

c) Digital images are collected from each slide and compiled into a short report so that participants each have a definitive account of the diatoms found in the sample. This means that ring-test slides will also form the basis of a useful reference collection for each participating laboratory.

d) The ring test co-ordinator provides feedback to any analysts who do not meet these criteria. In addition, these analysts shall provide their previous two operational slides to a mentor for audit in order to retain their accreditation (see below).

Audits involve the re-analysis of a slide by a second analyst. Audit results are presented as the difference in the national metric, as it is from this that ecological assessments are derived. In addition, Bray-Curtis similarity is calculated and this gives analysts supplementary information about those taxa which are responsible for differences. Preliminary studies established that replicate analyses by competent analysts should fall within ± 7 TDI units of each other; where the difference is greater, the auditor communicates the reasons for the deviations and the analyst sends another slide to be checked. Audits are performed for a number of reasons:

— as a preliminary test of competence;

— for re-accreditation of analysts who have taken breaks from routine sample analysis;

— for analysts whose ring-test results fall outside the limits of the exercise (see above); and

— for internal quality control. At present, the internal audit scheme only operates in Scotland. One in ten of each analyst's routine samples are selected at random and re-examined by a second analyst. Where the difference exceeds the limit for the exercise, the sample is sent to an experienced analyst for validation.

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