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ILNAS-EN 16695:2015

Water quality - Guidance on the estimation of phytoplankton biovolume

Qualité de l'eau - Lignes directrices pour l'estimation du biovolume des microalgues

Wasserbeschaffenheit - Anleitung zur Abschätzung des Phytoplankton-Biovolumens

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National Foreword

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European foreword

This document (EN 16695:2015) 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of an identical text or by endorsement, at the latest by March 2016 and conflicting national standards shall be withdrawn at the latest by March 2016.

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Introduction

The abundance or number of counting units of individual phytoplankton taxa does not necessarily reflect the real ratio of single taxa to the complete biomass of a phytoplankton community. Few big cells/counting units can contribute far more biomass to the system than many small ones. Hence, abundance data alone is often not an ideal measurement of population size. Biomass estimations give very important information for ecological studies, classification schemes and ecosystem modelling. Therefore, it is necessary to determine the biomass of phytoplankton taxa, particularly because phytoplankton delivers energy in the form of carbon, to other trophic levels of food webs. It is not possible to directly analyse the carbon content on the taxonomic level in natural phytoplankton samples, therefore the biovolume of the phytoplankton taxa is a suitable measure to determine the biomass of an ecosystem according to the taxonomic composition. Neither particle size analysis using laser analysis, nor flow cytometry, nor Coulter Counters, nor chemical analyses of chlorophyll-a concentration as well as total carbon allow statements on the taxon level. An estimation of the carbon content is possible using conversion factors (see Annex C).

Further, the biovolume is a quantitative basis for assessing hazards from those algae and cyanobacteria, which (can) contain noxious or toxic metabolites, and is used in combination with cell numbers or chlorophyll-a concentration within WHO guidelines and national regulations for risk assessments.

Up to now, various guidelines for estimating the biovolume of microalgae have been used in different national and international monitoring programs (e.g. [1], [2], [3], [4]). The main objective of this document is the standardization of the procedure for determining the phytoplankton biovolume in order to achieve comparability of data. For this reason, the estimation of the biovolume in phytoplankton samples in sedimentation chambers (according to Utermöhl) using an inverted microscope will be described in detail.

This European Standard is also applicable for image analysis of pictures derived from microscope and flow cytometry cameras. The use of a standard catalogue containing basic and some composed geometrical shapes is recommended. Of course, such a standard list will not reflect the variety of all naturally existing shapes and will not match the exact biovolume values of each taxon. It will always be a compromise between accuracy and efficiency. However, the usage of agreed geometrical shapes and the application of the relevant formulae will improve the comparability of phytoplankton data and will be an important step forward for the implementation of quality assurance measures in phytoplankton analysis.

1 Scope

This European Standard specifies a procedure for the estimation of biovolume of marine and freshwater phytoplankton taxa using inverted microscopy (Utermöhl technique according to EN 15204), in consideration of some heterotrophic protists (< 100 µm) that are not considered in routine zooplankton analysis and benthic microalgae, which can be found in pelagic water samples.

This European Standard describes the necessary methods for measuring cell dimensions and for the calculation of cell or counting unit volumes to estimate the biovolume in phytoplankton samples. This shall be done using harmonized assignments of geometrical shapes to avoid errors.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

EN 15972, *Water quality - Guidance on quantitative and qualitative investigations of marine phytoplankton*

EN 16698, *Water quality - Guidance on quantitative and qualitative sampling of phytoplankton from inland waters*

3 Terms and definitions

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

3.1
biomass
total mass of living organic matter within a system or taxon

3.2
biovolume
total volume of (living) organisms within a system or taxon

Note 1 to entry: The biovolume is usually expressed in cubic millimetres per litre (mm^3/l).

3.3
cell volume
counting unit volume
total volume of a single cell or one counting unit

Note 1 to entry: The cell volume or counting unit volume includes the cell wall (if existing) but excludes lorica and/or mucilaginous envelopes and cell surface structures such as spines, bristles and scales.

Note 2 to entry: The cell volume or counting unit volume is usually expressed in cubic micrometres (μm^3).

4 Principle

Generally, the estimation of the total or taxon specific biovolume in phytoplankton samples of natural communities or cultures is based on measurements of a representative number of individuals. By

multiplying the average or median cell or counting unit volume with the abundance, the total biovolume of each taxon in the sample is determined.

Three approaches are feasible:

1) **Estimation by representative measurement:** A representative number of individuals (in most cases single cells) or counting units of all recorded or dominating taxa is measured in each sample or a specified number of samples within a comparable series. These data are used to calculate the average or median cell or counting unit volume of each taxon using the applied geometrical formulae.

2) **Estimation using size classes based on representative measurements:** For taxa with a high variability in cell size (e.g. several diatoms, different stages in life cycle) reasonable size classes can be determined first, and then the individuals are assigned to both the relevant taxon and size class. Basis for the definition of the size classes are measurements in the same manner as described in (1).

3) **Estimation using standard volumes based on representative measurements:** A reasonable general standard cell or counting unit volume is defined for each taxon once. These standard values are determined by representative measurements and calculated by the formula of the assigned geometrical shapes as described in (1).

A geometrical shape shall be assigned to each taxon in all approaches to calculate the cell or counting unit volume. Thus, to harmonize these approaches the geometrical shapes are pre-assigned to all taxa (see Annex D). These shapes have been chosen to reflect the corresponding taxa shapes as accurately as possible, and to allow effective taxa measurement with little effort (i.e. with as few dimensions as possible; usually only two are necessary). Seventeen different geometrical shapes are utilized (for the catalogue of geometrical shapes see Annex A). If it is impossible to describe the actual shape of a taxon with a simple basic geometrical shape, composite shapes (e.g. cone with half sphere) are used. If the actual geometry of taxa does not fit exactly to the assigned shape, a “geometry correction factor” is used for the final cell or counting unit volume calculation.

Taxon lists describing the preferred geometrical shapes have been published before (see e.g. [1], [3], [4]), based on specific taxonomical levels or for particular areas. This guidance document provides harmonized geometrical shapes for phytoplankton organisms spread across European marine, brackish, and freshwater systems. Annex D contains an alphabetical list of genera with the assigned geometrical shapes. If there are divergent forms on species, subspecies, form, or variety level within a genus they are listed as well.

5 Equipment and preservatives

The following equipment is required for biovolume analysis of phytoplankton samples.

5.1 Inverted microscope equipped with a condenser featuring a numeric aperture (NA) of at least 0,5 and plan objectives with a NA of 0,9 or more allowing for total magnification between 63× and 400× at a minimum. The microscope should have binocular, bright field (additional phase contrast is useful), 10× or 12,5× eyepieces.

Though inverted microscopy is the recommended method for analysing of phytoplankton, conventional (non-inverted) compound light microscopes may also be used for measuring phytoplankton under some conditions.

5.2 Calibrated object micrometre.

5.3 Eyepiece (ocular) micrometre.

5.4 Counting-graticule.

5.5 Sedimentation chambers according to EN 15204.

5.6 Image analysis software, if available.

5.7 Sampling bottles according to EN 15204.

5.8 Preservatives, acidic Lugol's iodine and/or alkaline Lugol's iodine according to EN 15204.

6 Procedure

6.1 Sampling and sample preparation

The sampling and determination of phytoplankton abundance and composition is a precondition for the calculation of the biovolume of a phytoplankton sample. Sampling shall be carried out according to EN 16698 for freshwater samples and EN 15972 for marine samples. For counting and species determination, see EN 15204.

The dimensions needed for the biovolume estimation of the relevant phytoplankton taxa are analysed in sedimentation chambers, which are prepared in the same manner as for counting and species determination (see EN 15204), using an inverted microscope and an eyepiece micrometre or image analysis software.

For specific scientific purposes, measurements can be carried out also with a conventional (non-inverted) compound light microscope.

6.2 Calibration of the eyepiece micrometre, counting-graticule and image analysis software

The required dimensions for estimation of the cell or counting unit volume shall be measured using an eyepiece (ocular) micrometre or an image analysis software. For the application of size classes a calibrated counting-graticule can also be used.

Prior to measurement, all systems shall be calibrated with a calibrated object micrometre for every microscope and all objectives and eyepieces used.

The scale of commercially available calibrated object micrometres has a length of 1 mm (or 2 mm) where the millimetre is divided into 100 equal parts. The distance between the graduation lines is 10 µm. By aligning the scale of the eyepiece micrometre with the scale of the object micrometre or the grid boxes of the counting-graticule, the scale value (S) or conversion factor of the eyepiece micrometre can be determined for each magnification as follows:

$$S = \frac{n_{\text{obj}} \times 10}{n_{\text{eye}}} \quad (1)$$

where

S is the scale value (conversion factor) for the eyepiece micrometre in micrometres (µm);

n_{obj} is the number of graduation lines of the object micrometre;

n_{eye} is the number of graduation lines of the eyepiece micrometre or the number of grid boxes of the counting-graticule.