SLOVENSKI STANDARD
SIST EN 16161:2013

01-januar-2013

Kakovost vode - Navodilo za uporabo absorpcijskih tehnik in vivo za oceno koncentracije klorofila a v morskih in celinskih vodah

Water quality - Guidance on the use of in vivo absorption techniques for the estimation of chlorophyll-a concentration in marine and fresh water samples

Wasserbeschaffenheit - Anleitung für die Anwendung der in-vivo-Absorption zur Abschätzung des Chlorophyllgehaltes in Meer- und Süßwasser

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Qualité de l'eau - Lignes directrices sur l'utilisation de techniques d'absorption in vivo pour l'estimation de la concentration de chlorophylle a dans des échantillons d'eau de mer et d'eau douce

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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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2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

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EUROPEAN STANDARD

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English Version

Water quality - Guidance on the use of in vivo absorption techniques for the estimation of chlorophyll-a concentration in marine and fresh water samples

Qualité de l'eau - Lignes directrices sur l'utilisation des techniques d'absorption in vivo pour l'estimation de la concentration de chlorophylle-a dans les eaux douces et eaux marines

Wasserbeschaffenheit - Anleitung für die Anwendung der in-vivo-Absorption zur Abschätzung der Chlorophyll a-Konzentration in Meer- und Süßwasser

This European Standard was approved by CEN on 17 May 2012.

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COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 16161:2012) 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of an identical text or by endorsement, at the latest by January 2013, and conflicting national standards shall be withdrawn at the latest by January 2013.

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Introduction

Surveys of chlorophyll and nutrient concentration are fundamental descriptors of primary productivity and eutrophic threat in coastal and inland waters.

Chlorophyll-a concentration can be determined by sampling and laboratory analysis using the techniques described in ISO 10260. Achieving consistent results with this technique requires careful attention during the various steps of the process commonly used, such as during sampling, transport, filtering, freezing, storage and extraction and subsequent pigment estimation.

The *in vivo* technique described here can be applied to surveys where a rapid non-destructive and repeatable measurement capability is required. It can be used either in the field or laboratory. No chemicals are required. Utilised in association with other methods of chlorophyll-a determination such as ISO 10260, HPLC pigment analysis and chlorophyll fluorescence measurements techniques, it can help identify sources of inconsistency or be used as an alternative technique in its own right. As chlorophyll-a estimates can be achieved in times as short as one minute, the technique can enhance surveying capability considerably.

This standard describes procedures to implement and verify performance.

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1 Scope

This European Standard provides guidance in the use of *in vivo* absorption techniques to quantify chlorophyll-a concentration in marine and fresh waters.

This European Standard is comprised of the following:

— definition of the equipment requirement;

— *a priori* data and mathematical tools;

— recommendations for verification of measurement system performance and consideration of factors that can influence measurements;

— listing of the procedures to be implemented.

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.

ENV 13005, *Guide to the expression of uncertainty in measurement*

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3 Terms and definitions

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

3.1
absorption coefficient
a
natural logarithm of the ratio between the light intensity entering and corresponding intensity emerging directly through a sample of water divided by the sample path length (in metres) in cases where the scattering of light is negligible

Note 1 to entry: The unit is m^{-1}.

Note 2 to entry: A spectrophotometer often gives the Log_{10} of the ratio in place of the natural logarithm.

3.2
extinction
c
sum of losses of directly transmitted light by absorption and scattering

Note 1 to entry: The unit is m^{-1}.

Note 2 to entry: The extinction c is related to absorption a and scattering b, by $c = a + b$.

3.3
extractive photometric
EP
method of chlorophyll concentration estimation involving extraction and absorption measurement

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3.4
***in vivo* photometric**
IVP
method of assessing chlorophyll-a concentration through the use of *in vivo* spectral photometry

3.5
package effect
flattening of a spectral absorption feature arising from excessive absorbing molecule concentration within cells

3.6
resolution
width at half height of the instrument response function

3.7
scattering coefficient
b
natural logarithm of the ratio between the light intensity entering and corresponding intensity emerging directly through a sample of water divided by the sample path length (in metres) in cases where the absorption of light is negligible

Note 1 to entry: The unit is m^{-1}.

3.8
spectrum
set of data of a sample taken over a defined wavelength range and by a defined resolution

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3.9
wavelength range
range from minimum to maximum wavelength over which a spectrum is described

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4 Principle

The *in vivo* photometric absorption technique (IVP) is based on:

a) the additive nature of absorption of individual constituents within a suspension;

b) the use of a priori knowledge about the absorption features of chlorophyll-a in the wavelength area of approximately 675 nm;

c) the absence of other components interfering with spectral features of chlorophyll-a in this region;

d) the use of a measurement cell (a cuvette or other sample receptacle) of sufficient length and spectrophotometer of sufficient performance to enable the absorption feature of chlorophyll-a to be identified at the concentration levels required;

e) the availability of a suitable algorithm to identify and quantify the distinctive chlorophyll-a absorption feature within a spectral absorption measurement.

5 Apparatus

5.1 Spectrophotometer, or equivalent, with the capability to determine the absorption spectrum of an *in situ* sample of water.

The spectral measuring instrument shall be capable of measuring the absorption of the sample in a range between 600 nm and 750 nm with a resolution better than 10 nm. The data capture is carried out in measuring intervals of 5 nm or smaller.

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NOTE For example, a spectrophotometer with 2 nm resolution requires the ability to sense changes in absorption around 675 nm of 0,020 3 m^{-1} in order to sense 1 µg/l of chlorophyll-a.

5.2 Datum, consisting of the unit response spectrum for chlorophyll-a absorption $a_s(\lambda)$ in the region around 675 nm for the spectrophotometer in use.

See Annex B for suggested methods of determining this.

5.3 Mathematical routine, capable of determining the quantity C of unit chlorophyll-a spectra present in the sample spectrum.

6 Procedure

6.1 Calibration

The reference spectrum should be recorded with a sample of water which is free of constituents with absorption effects likely to mimic or interfere with chlorophyll-a absorption spectral features in the 650 nm to 710 nm region. This measurement should be stored as a system reference spectral signal of the water $I_w(\lambda)$. The temperature of the reference water sample should not differ more than around 10 °C from that of the samples to avoid affecting chlorophyll estimation results at the 1 µg/l level.

If the same measurement cell is used for successive samples, the measuring chamber should be flushed to remove influences of previous measurements before the next measurement (see 6.3). This is particularly necessary in cases where a flow-through measurement cell is utilised. Persistent positive residual chlorophyll-a measurements on clean water samples indicate window fouling by chlorophyllous material. Either the measurement cell should be cleaned or the effects of the persistent fouling should be zeroed out by taking a new clean water reference spectrum.

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Water free of chlorophyll-a can be generated by filtering drinking water through a pore size of 0,1 µm. For the measurement of samples with high content of dissolved salts or colour (especially high content of humic substances), a representative water sample can be filtered for use as reference.

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When a measurement cell is emptied there is often a residue. In practice, the number of flushes recommended should be sufficient to bring verification of zero measurements to within two standard deviations of zero. If a significant positive residue persists, this might indicate window fouling and either a new reference clean water sample should be taken or the measurement cell should be cleaned.

EXAMPLE A sample of clean water free of chlorophyll inserted after a measurement of a sample with 100 µg/l of chlorophyll-a may record a residual chlorophyll-a concentration of 2 µg/l, or a 2 % residual. Two flushes should reduce this to 2 % of 2 % or 0,04 % and three flushes to 0,008 %.

The effect of temperature difference on the system algorithm can be checked by repeating measurements on one sample using different reference water or sample water temperatures. Such effects arise because of small changes in the absorption of pure water with temperature. These changes are well described in the literature [15] and can influence the curvature of an absorption spectrum near 675 nm.

The influence of the temperature and the salinity differences between the spectrum of the sample and reference spectrum shall be considered if concentrations around or below 1 µg/l need to be determined accurately. These changes are well described in the literature [15] and can influence the shape of an absorption spectrum near 675 nm.

6.2 Blank Measurement

For any instrument system, the standard deviation of the determination of C in a series of measurements on a clean water sample should be determined by statistical methods. This will indicate the contribution of instrument and algorithm fitting noise to the determination of minimum detectable chlorophyll concentration. For each new reference water, a second measurement of that water used as a sample to determine estimated chlorophyll C should be made. This estimate should not exceed $\pm$ two standard deviations of zero for the

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instrument. If this is not the case, flushing, calibration (6.1) and blank measurement (6.2) should be repeated until this condition is fulfilled. Further clean samples based on the same reference water should also meet this condition in > 95 % of the measurements.

6.3 Sample measurement

Load and measure the sample. After filling the well-mixed sample in the measuring chamber, the transmitted light signal $I_s(\lambda)$ should be measured within one minute to avoid mistakes which are caused by sedimentation of algal cells

Care should be taken to include appropriate flushing operations when successive samples show substantially decreasing chlorophyll-a concentrations or turbidity effects. The chlorophyll-a estimate should be calculated from $I_s(\lambda)$,$I_w(\lambda)$, and d to yield $a_s(\lambda)$ (see Formula (1)). When carried out using a curve fitting algorithm, the system unit chlorophyll-a response should be used and a modelled absorption should be adjusted to fit the measurement $a_s(\lambda)$. The value of C, the concentration of chlorophyll-a in the model at time of fit, is the system output. The sample spectrum and other information derived from it (dissolved organic matter, DOM, total visible absorption, etc.) can then be computed and recorded.

7 Calculation and Expression of Results

7.1 General

The inclusion of some or all of the effects of scattering can be tolerated. A sample absorption spectrum should be recorded according to Formula (1):

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$$a_s(\lambda) = (1/d)(-\ln(I_s(\lambda)/I_w(\lambda))) \quad (1)$$

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where

$a_s(\lambda)$ is the sample absorption spectrum (m^{-1});

I_s is the signal observed through the sample;

I_w the signal observed through clean water, free of chlorophyll;

d is the optical path length of the light through the sample, in metres (m).

7.2 Datum

The datum describes the unit response spectrum for chlorophyll-a absorption $a_{chl}(\lambda)$ in the region around 675 nm for the spectrophotometer in use. See Annex B for suggested methods of determining this and Annex D for examples.

A multiple of the datum of chlorophyll-a should be recorded according to Formula (2):

$$a_{chl}(\lambda) = a_0 + a_1(\lambda - \lambda_0) \quad (2)$$

where

$a_{chl}(\lambda)$ is the unit response spectrum for chlorophyll-a absorption effective over a range of wavelengths from approximately 600 nm to 710 nm.

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7.3 Mathematical routine

The mathematic routine used shall be capable of determining the quantity C of unit chlorophyll-a spectra present in the sample spectrum. For example, a minimisation routine capable of finding the best fit between a sample absorption spectrum $a_s(\lambda)$ and the model spectrum $a_0 + a_1(\lambda - \lambda_0) + c \times a_{chl}(\lambda)$, by varying C until sample and model spectra have minimum differences over the range 650 nm to 710 nm (see Annex E).

7.4 Other factors influencing the chlorophyll-a estimation

The following differences that may arise should be assessed:

a) breakdown pigments with similar spectral features;

b) the package effect;

c) the presence of other pigments whose absorption features extend to some extent into the 675 nm region; principally phycocyanin and chlorophyll-b.

The effects that these factors can have on a measurement are described in Annex C.

NOTE This method yields an estimate based on the chlorophyll-a-like absorption feature at 675 nm as it appears *in vivo*. This is not necessarily the same as the chlorophyll-a absorption effect produced by the chlorophyll-a molecules alone when extracted from the *in vivo* cells. While this *in vivo* estimate is a good measure of the effective solar harvesting capability of the chlorophyll-a within the cell at the time of measurement, the differences that may arise when compared with extracted chlorophyll-a estimates should be clearly understood.

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8 Quality Assurance

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8.1 Repeatability

The absorption characteristics may change if the samples remain in the measuring chamber for a longer time (e.g., with multiple measurements, by sedimentation, agglomeration or buoyancy of algae cells). Therefore, the measuring cell should be equipped with a stirrer; or else the measuring chamber shall be filled again after each single measurement.

8.2 Uncertainty

The measurements should be accompanied by a statement of uncertainty, prepared by taking full account of natural factors which may influence or interfere with the estimation.

Uncertainty estimation requires clear identification of:

a) the measurand, and

b) the uncertainty sources in accordance with ENV 13005.

Once the uncertainty sources are identified, they should be quantified and combined into a total uncertainty.

NOTE 1 Further useful guides to the estimation of uncertainty can be found in the bibliography, [13] and [14].

The measurand here is the chlorophyll-a estimate. Tracing backwards, the main sources of uncertainty are:

1) the uncertainty of the performance of the mathematical algorithm in determining the concentration C of chlorophyll-a, i.e. in determining the amplitude of the system unit chlorophyll-a response present in the measured absorption spectrum;

2) the uncertainty in determining the system unit chlorophyll-a response from the a priori unit chlorophyll-a response (Annex A) which is used in 1 above;