SLOVENSKI STANDARD
SIST EN 17899:2024

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Kakovost vode - Spektrofotometrijsko določevanje klorofila-a po ekstrakciji z etanolom za rutinski monitoring kakovosti vode

Water quality - Spectrophotometric determination of chlorophyll-a content by ethanol extraction for the routine monitoring of water quality

Wasserbeschaffenheit - Spektrophotometrische Bestimmung des Chlorophyll-a-Gehalts durch Ethanolextraktion für das Routinemonitoring der Wasserqualität

Qualité de l'eau - Détermination spectrophotométrique de la teneur en chlorophylle a par extraction à l'éthanol pour la surveillance de routine de la qualité de l'eau

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English Version

Water quality - Spectrophotometric determination of chlorophyll-a content by ethanol extraction for the routine monitoring of water quality

Qualité de l'eau - Détermination spectrophotométrique de la teneur en chlorophylle a par extraction à l'éthanol pour la surveillance de routine de la qualité de l'eau

Wasserbeschaffenheit - Bestimmung des Chlorophyll-a-Gehalts durch Ethanolextraktion für die routinemäßige Überwachung der Wasserqualität

This European Standard was approved by CEN on 10 June 2024.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (EN 17899:2024) has been prepared by the 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of an identical text or by endorsement, at the latest by January 2025, and conflicting national standards shall be withdrawn at the latest by January 2025.

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

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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Introduction

Chlorophyll-a is the most common essential photosynthetic pigment present in photoautotrophic plankton organisms. It is the main component of the dynamically regulated photosystem of these organisms, in which other accessory pigments are involved, some of which are chemically very similar to chlorophyll-a.

The chlorophyll-a content depends on the species composition of the phytoplankton, the time of day and season, the place and the depth of sampling. It is also suitable for quantifying the change in the algal biomass (cell proliferation) in biological tests to check the toxicity of substances dissolved in water.

The chlorophyll concentration of a water sample can provide information about the trophic state of a water body. It is used as an easily determinable measure of the phytoplankton biomass and is a key variable in many trophy scoring systems. Even if this value cannot be used as an absolute measure for the phytoplankton biomass, the determination of the chlorophyll-a concentration together with other biomass and bioactivity parameters provides information about the quantitative occurrence and the potential metabolic performance of the phytoplankton in waters.

Due to the sensitivity of chlorophyll to light, acids and enzymes, there is currently no universally applicable routine analytical method that enables an accurate, artefact-free and at the same time simple determination of the chlorophyll-a content in water samples containing phytoplankton. The extractive, spectrophotometric method described in this document therefore provides an operationally defined value. As extracting agent hot ethanol is used. Various other extractants (e.g. acetone or methanol) are described in the literature, but these may have lower extraction efficiency or are toxicologically problematic.

WARNING — Persons using this document should be familiar with usual laboratory practice. This document 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 safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

Annexes A, B, C and D of this document are for information only.

1 Scope

This document describes a spectrophotometric method for determining the chlorophyll-a content corrected for phaeopigments as a measure of the amount of phytoplankton for all types of surface water including marine water. The determination limit is usually 2 µg/l to 5 µg/l and is calculated by each laboratory individually. It can be as low as 0,5 µg/l using 2 l of sample (or even more) and a 50 mm cuvette.

NOTE In some measurement programs like marine studies on time series data and ecological status/classification no correction for phaeopigments is used and acidification is omitted, e.g. as recommended by OSPAR.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples (ISO 5667-3)*

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
chlorophyll-a
natural plant pigment that is present in phytoplankton, most important photosynthetic pigment of photoautotrophic organisms

Note 1 to entry: For the purposes of this document, the pigment whose concentration can be determined by measuring the absorbance at 665 nm in an ethanolic solution corrected for phaeopigments.

3.2
phaeopigments
collective term for chlorophyll breakdown products, which do not contain any magnesium

EXAMPLE Phaeophytin is a phaeopigment.

3.3
phytoplankton
community of free-living, suspended, mainly photosynthetic organisms in aquatic systems comprising cyanobacteria and algae

3.4
accessory pigments
auxiliary pigments that support photosynthesis

EXAMPLE Carotinoids, phycobilins, chlorophyll-b and chlorophyll-c.

4 Principle

The determination of the chlorophyll-a concentration according to this document is based on the hot ethanolic extraction of a water sample's filter residue and the subsequent absorbance measurement at 665 nm, whereby phaeopigments are also recorded. After the quantitative conversion of the chlorophyll-a into phaeopigments by means of acidification and renewed measurement at 665 nm, the chlorophyll-a concentration of the water sample corrected for phaeopigments is calculated with the formula defined by Lorenzen (see Reference [12]).

5 Interferences

Macroscopic parts of plants in the water sample (e.g. duckweed, drifted benthic filamentous algae, torn off macrophyte parts) as well as large zooplankton can falsify the measurement result. They shall be removed from the water sample, if they should not expressly be included.

In the case of water samples with a pH value of < 5, chlorophyll degradation by acid occurs in the filter residue. To prevent this, the pH value shall be neutralized by rinsing the filter with about 50 ml of a 0,1 molar ammonium acetate solution (see Reference [8]). This should be done shortly before the end of the filtration, when there are only a few millilitres of sample left in the filter funnel.

Chlorophyll is very sensitive to light, especially in extracted solution. If the extract is exposed to direct sunlight or bright artificial light, the pigment is destroyed photochemically.

Changes in the concentration of the extractant due to evaporation are to be avoided by working with firmly sealable extraction vessels.

During the extraction and homogenization of the filters, turbidity may occur which impairs the precision of the photometric measurement; it shall be removed by filtration or centrifugation (see 8.4). The same applies to turbidity in the extract caused by other reasons (e.g. after acidification).

Pigments of (rarely occurring) autotrophic bacteria (e.g. chlorobium-chlorophyll, "bacterioviridin") may influence the determination of chlorophyll-a.

6 Reagents

Use reagents of an analytical pure quality and deionized or destilled water.

6.1 Ethanol, C_2H_5OH, volume fraction 96 %.

Pure ethanol and ethanol denatured with methanol or methyl ethyl keton (MEK) do not interfere with the determination of chlorophyll-a and can also be used.

6.1.1 Ethanol, C_2H_5OH, volume fraction 90 %.

Add 60 ml of water to 900 ml of ethanol 96 % (6.1).

6.2 Hydrochloric acid, HCl, 12 mol/l, ρ = 1,19 g/ml.

6.2.1 Hydrochloric acid, HCl, 0,4 mol/l.

Add 10 ml of concentrated hydrochloric acid (6.2) to 290 ml of water.

6.3 Ammoniumacetate solution, $C_2H_7NO_2$, 0,1 mol/l.

7 Apparatus

Special attention using laboratory equipment and devices is needed to minimize the influence of UV light.

7.1 Vacuum pump, preferably diaphragm vacuum pump with barometer, with suction bottle with a volume of 2 l to 5 l, with silicone or rubber stopper.

7.2 Filtration apparatus for water samples with tightly closing funnel, preferably for filter diameters of 45 mm to 55 mm.

7.3 Filtration apparatus for extracts, e.g. Witt's pot to accommodate a volumetric flask, 200 mm high and internal width 150 mm, with side tube for hose attachment, flat ground lid with central tube, rough ground for rubber stopper connection, and filter attachment for round filters with low dead volume (e.g. perforated plate as filter support).

NOTE The filtration apparatus can be protected from excessive incidence of light by means of an opaque film or by painting it - with the exception of a viewing window.

7.4 Glass fibre filter, made of borosilicate glass, without binding agent, separation efficiency > 98 % for particles > 0,7 µm, recommended diameter 45 mm to 55 mm.

7.5 Filters for clearing of extracts, ash-free, slow-filtering paper filters with high separation efficiency or cellulose acetate membrane filters with a pore size of 0,45 µm.

Cellulose nitrate filters cannot be used because of losses of chlorophyll-a.

7.6 Extraction vessel, protected from light, tightly closable, e.g. wide neck bottles or vials made of (amber) glass with screw cap, nominal volume approx. 30 ml to 100 ml. If clarification of the extract is done by centrifugation, glass vials or centrifuge tubes should be used.

7.7 Centrifuge, with an acceleration of at least 3000 *g*, suitable for glass vials or centrifuge tubes with tightly fitting screw caps, nominal volume 15 ml to 50 ml; light protected (e.g. made of brown polypropylene), also transparent when working in darkened rooms.

7.8 Shaking water bath, set at (75 ± 1) °C.

7.9 Spectrophotometer, suitable for absorbance measurements at 665 nm and 750 nm, with the following recommended performance characteristics: spectral bandwidth ≤ 2 nm, photometric accuracy ≤ ± 0,005 at 1 abs. (decadic absorbance), wavelength accuracy ≤ ± 1 nm.

NOTE Suitable devices are in particular split-beam, dual-beam or reference beam path spectrophotometers.

Devices with an integrated self-test to check for their correct function are recommended.

7.10 Rectangular cuvette, with a path length of 10 mm to 50 mm (or even 100 mm can be used).

NOTE In most cases, the use of 50 mm cuvettes will be suitable.

7.11 Freezer, with a temperature lower than −18 °C.

7.12 Freezer, with a temperature lower than −80 °C.

7.13 pH meter with electrode, suitable for pH measurements in alcohol.