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**Kakovost vode - Določevanje učinkov strupenosti sedimenta na rast *Myriophyllum aquaticum***

Water quality - Determination of the toxic effect of sediment on the growth behaviour of *Myriophyllum aquaticum*

**iTeh STANDARD PREVIEW**

Qualité de l'eau - Détermination de l'effet toxique des sédiments sur la croissance de *Myriophyllum aquaticum*

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# INTERNATIONAL STANDARD

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## **Water quality — Determination of the toxic effect of sediment on the growth behaviour of *Myriophyllum aquaticum***

*Qualité de l'eau — Détermination de l'effet toxique des sédiments sur  
la croissance de *Myriophyllum aquaticum**

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## ISO 16191:2013(E)

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2, [www.iso.org/directives](http://www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received, [www.iso.org/patents](http://www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

The committee responsible for this document is ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

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## Introduction

The contact test with *Myriophyllum aquaticum* described in this International Standard allows the measurement of responses of the plant to dissolved and particle-bound substances present in sediment samples within 10 d (References [3][4][5][6][7][8]).

The test plant, *Myriophyllum aquaticum* (Velloso) Verdcourt (parrot feather), is a dicotyledonous macrophyte. It is a native of the Amazon River in South America, but it has naturalized worldwide, especially in warmer climates. It has been introduced worldwide for use in indoor and outdoor aquaria. For its use as test organism, its capability for emerged growth (no additional liquid as supernatant is needed), its strong regeneration potential, and its vegetative growth are harnessed in the contact test. Furthermore, *Myriophyllum aquaticum* grows without generating side shoots during the test period, which facilitates handling in the laboratory. However, it should be ensured that no live plant material is lost from the laboratory.

*Myriophyllum aquaticum* can be affected by phytotoxic substances present in sediments (e.g. dredged material). The subsequent inhibition of growth is calculated from the parameter (fresh mass) by a number of defined calculation methods.

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# Water quality — Determination of the toxic effect of sediment on the growth behaviour of *Myriophyllum aquaticum*

**WARNING** — Persons using this International Standard should be familiar with normal laboratory practice. This International 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 safety and health practices and to ensure compliance with any national regulatory conditions. It shall be ensured that no plant material can elude the laboratory.

**IMPORTANT** — It is absolutely essential that tests conducted according to this International Standard be carried out by suitably trained staff.

## 1 Scope

This International Standard specifies a method for determining the toxicity of environmental samples on the growth of *Myriophyllum aquaticum*. The method described is applicable to natural fresh water sediment and artificial sediment.

## 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.

ISO 5667-15, *Water quality — Sampling — Part 15: Guidance on the preservation and handling of sludge and sediment samples*

ISO 5667-16, *Water quality — Sampling — Part 16: Guidance on biotesting of samples*

ISO 10523, *Water quality — Determination of pH*

ISO 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO 20079, *Water quality — Determination of the toxic effect of water constituents and waste water on duckweed (Lemna minor) — Duckweed growth inhibition test*

OECD 218, *OECD Guidelines for the testing of chemicals — Sediment-water Chironomid toxicity test using spiked sediment*

## 3 Terms and definitions

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

### 3.1

#### **artificial sediment**

defined artificial sediment

[SOURCE: ISO 10872:2010, <sup>2</sup> definition 3.3, modified]

Note 1 to entry: See 6.9.

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## 3.2

**chlorosis**

loss of pigments (yellowing of plant tissue)

[SOURCE: ISO 20079:2005, definition 3.3, modified]

## 3.3

**control sample**

artificial sediment pre-treated according to the need of this test that serves as negative control to which the effect in the respective test material is compared

[SOURCE: ISO 10872:2010, definition 3.6, modified]

## 3.4

**effective concentration**

$E_rC_x$

concentration of a substance in a test sample ( $EC_x$ ) at which an effect of  $x$  % is measured, if compared to the control

[SOURCE: ISO 20079:2005, definition 3.9, modified]

Note 1 to entry: To unambiguously denote an EC value deriving from growth rate, it is proposed to use the symbol " $E_rC_x$ ".

## 3.5

**emersed growth**

morphological habitus of aquatic macrophytes, growing above the water surface

## 3.6

**head-whorl**

apical part of a *Myriophyllum* plant

Note 1 to entry: See [Figure A.1](#).

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## 3.7

**necrosis**

localized dead plant tissue (i.e. brown or white)

[SOURCE: ISO 20079:2005, definition 3.16, modified]

## 3.8

**nutrient solution**

solution of nutrients and micronutrients in water which are essential for the growth of *Myriophyllum*

[SOURCE: ISO 20079:2005, definition 3.17, modified]

## 3.9

**pre-culture**

culture of *Myriophyllum aquaticum* used for acclimatization of test plants to the test conditions and for the growing of the plants to be used as whorls at test start

[SOURCE: ISO 20079:2005, definition 3.19, modified]

Note 1 to entry: See [Figure A.2](#).

## 3.10

**replicate**

one of a selected number of test vessels (containing sample material from one sample and test organisms)

Note 1 to entry: Each vessel is tested.

Note 2 to entry: The replicates mentioned in this International Standard contain sample material (e.g. natural sediment) and three whorls of *Myriophyllum aquaticum*.

**3.11****test sample**

discrete portion of a sample (e.g. sediment or artificial sediment)

[SOURCE: ISO 10872:2010, [2](#) definition 3.14, modified]

**3.12****whorl**

arrangement of leaves that radiate from a single point and surround the stem

Note 1 to entry: See [Figure A.1](#).

**4 Principle**

*Myriophyllum aquaticum* whorls are exposed to test samples over a period of 10 d. The growth of *Myriophyllum aquaticum* in a test sample is compared with its growth in the control sample. Phytotoxic effects are quantified as growth inhibition (%) relative to the control growth.

**5 Interferences**

In case of problems with *Myriophyllum* control growth using artificial sediment, the respective components should be checked, first to exclude contamination with, for example, heavy metals (kaolin) or suitability of peat (if the recommended peat is not used).

**6 Reagents**

Use, as far as possible, reagents of recognized analytical grade.

**6.1 Water**, distilled or deionized water or water of equivalent purity, conductivity < 10 µS/cm.

**6.2 Kaolin clay**, kaolin powder (CAS RN 1332-58-7).

**6.3 Calcium carbonate**, CaCO<sub>3</sub> powder (CAS RN 471-34-1).

**6.4 Quartz sand**, average grain size 170 µm (see [Annex C](#)).

**6.5 Reference substance**, 3,5-dichlorophenol [C<sub>6</sub>H<sub>4</sub>OCl<sub>2</sub> (purity ≥ 99 %), CAS RN 591-35-5].

**6.6 Nutrient solution**, use Steinberg medium as specified in [Annex B](#).

**6.7 Peat**, Sphagnum peat (e.g. Lithuania peat), H2-H5, fine (grain size ≤5 mm) (see [Annex C](#)).

**6.8 Peat powder**, dry peat ([6.7](#)) for 7 d at room temperature.

Spread the peat on shallow trays, and turn the peat every 2 d to 3 d. Then grind the peat and sieve it through a 0,5 mm sieve. Determine dry mass of the peat powder by drying a small sub-sample at 60 °C for 3 h in four aliquots, and determine the dry mass by re-weighing until constant mass (see ISO 11465). Store the peat powder in airtight vessels until use. Note down the dry mass on the vessel.

**6.9 Artificial sediment**, see [Table 1](#)