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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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Foreword

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

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* (Vellosco) 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 emersed 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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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,[2] definition 3.3, modified]

Note 1 to entry: See 6.9.

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,[2] 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.

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, $CaCO_3$ 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 [$C_6H_4OCl_2$ (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

Table 1 — Dry constituents for composition of the artificial sediment

Constituents	% of sediment dry mass	Characteristics
Peat powder	5	see 6.8
Quartz sand	74	average grain size 170 µm
Kaolin clay	20	powder
$CaCO_3$ powder	1	pro analysis

6.9.1 Artificial sediment as control sample.

Preparation of the artificial sediment as control sediment is described below (6.9.1.1 to 6.9.1.3). The dry constituents for preparation of the artificial sediment may be stored separately in closed, airtight vessels in a dry and dark place at room temperature for at least 6 months.

NOTE These requirements (6.9.1.1 to 6.9.1.3) are carried out for establishing stable ambient conditions in the sediment and avoiding separation of the sediment components during the test.

6.9.1.1 Preparation of peat suspension.

Take the required amount of peat powder (6.8, Table 1) and $CaCO_3$ powder (see Table 1) and add nutrient solution (6.6) until the suspension can be stirred easily (at maximum 50 % of total sediment dry mass). Stir carefully. Keep the peat suspension for 3 d to 4 d with continuous gentle stirring at room temperature to stabilize pH. Afterwards, measure pH of the suspension and adjust if necessary to 6,7 ± 0,5 by adding $CaCO_3$ powder.

NOTE Experience has shown that the pH is at 6,7 ± 0,5; therefore, no further addition of $CaCO_3$ is usually necessary.

6.9.1.2 Addition and blending of sediment components.

Mix the peat suspension with the other constituents (quartz sand and kaolin clay, see Table 1) to obtain a homogeneous sediment. Measure the pH of the final mixture again and adjust it to 7,0 ± 0,5 by adding $CaCO_3$ powder if necessary.

NOTE Experience has shown that the pH is at 7,0 ± 0,5; therefore, no further addition of $CaCO_3$ is usually necessary.

6.9.1.3 Conditioning of the mixed sediment suspension.

Add nutrient solution (6.6) as supernatant to the mixed sediment (6.9.1.2) in the ratio: 1 part (mass) mixed sediment plus maximum 0,5 parts (volume) nutrient solution. Condition this mixture for 7 d to 9 d under exposure conditions (see 10.5) to establish a stable microbial component and to avoid separation of the sediment components during the test. Remove the supernatant of nutrient solution carefully after 7 d to 9 d. The sediment is ready for instant use.

NOTE Experience has shown that the artificial sediment can be stored at 4 °C to 8 °C in the darkness for 14 d.

6.9.2 Artificial sediment for pre-culturing.

Mix the dry sediment constituents (see Table 1) and shake the dry mixture for 2 h to 3 h in a rotary shaker at room temperature. The dry sediment powder mixture can be maintained without restraint in an airtight vessel in a dark and dry place at room temperature.

Measure 125 g dry sediment powder mixture into a 1 000 ml pre-culture vessel, add (65 ± 5) ml nutrient solution (6.6), and stir carefully until it is homogeneous. The sediment suspension should be muddy.

Condense the sediment by knocking the vessels on the table to eliminate air bubbles and cavities within the sediment matrix.

NOTE Growing of the pre-cultures (see 10.1) in artificial sediment for control samples (6.9.1) or in artificial sediment for pre-culturing (6.9.2) has no significant influence on the results of a subsequent test; therefore, both artificial sediments (6.9.1 and 6.9.2) are suitable as pre-culturing sediment.

7 Apparatus

Usual laboratory equipment and, in particular, the following:

7.1 Autoclave.

7.2 Cylindrical or conical vessels, plastic or glass beakers, crystallizing dishes [e.g. for the pre-culture: diameter bottom 10 cm, diameter top 13 cm, height 11 cm; for the test: 250 ml glass beakers, low form; see Figures A.2 b) and A.3 b)].

7.3 Drying oven, approximately 105 °C.

7.4 Temperature-controlled incubator with constant illumination, e.g. climate chamber.

7.5 Light meter, to measure photosynthetically active radiation (PAR), within the photosynthetic range 400 nm to 700 nm with a spherical quantum sensor.

7.6 pH meter.

7.7 Precision balance, required accuracy of 0,1 mg.

7.8 Rotary shaker.

7.9 Scalpel or scissors.

7.10 Sieve, stainless steel, mesh size 0,5 mm.

7.11 Translucent lids, glass or plastic, with openings (e.g. holes) to allow air and humidity exchange [see Figures A.2 b) and A.3 b)].

7.12 Tweezers.

7.13 Stirrer.

7.14 Glass electrode, to measure pH values of aqueous solutions and sediments.

7.15 Grinder, to pulverize peat after drying (e.g. blender).

7.16 Mortar, to homogenize sediments after drying (see Annex D).

7.18 Fume hood (see Annex D).