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Water quality — Determination of toxicity of fresh water sediments using *Hyalella azteca*

Qualité de l'eau — Détermination de la toxicité des sédiments d'eau douce vis-à-vis de Hyalella azteca

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Foreword

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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 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

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

Introduction

Sediment in the aquatic environment serves as a reservoir for agricultural, industrial, and municipal contaminants. Contaminated sediment adversely affects the benthic community directly and acts as a source of contamination for the overlying water, often negatively impacting pelagic communities as well. Sediment toxicity tests are used globally to determine and monitor the toxic effects of discrete substances or complex mixtures that might be harmful to indigenous life in the aquatic and benthic environments. This International Standard outlines procedures for conducting 14 d and/or 28 d tests for sediment toxicity, using the fresh water amphipod *Hyalella azteca*. The biological end points for the tests include mortality and growth.

Water quality — Determination of toxicity of fresh water sediments using *Hyalella azteca*

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.

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

1 Scope

This International Standard specifies a method for the determination of toxicity to young *Hyalella azteca* in whole sediment based on survival and growth inhibition after 14 d and/or 28 d.

The method is applicable to

a) samples of contaminated whole fresh water sediment,

b) chemical, industrial, or municipal sludge, or other solid wastes that may combine with fresh water sediments, and

c) chemicals or preparations spiked into clean sediment.

This International Standard is applicable to the testing of sediment samples from the fresh water environment. *Hyalella azteca* can be used in the testing of brackish waters up to a maximum of 15 ‰, with careful acclimation. This International Standard is not applicable to the testing of sediment samples from the marine and estuarine environment with a salinity of > 15 ‰.

This method is a 14 d and/or 28 d survival-and-growth test applicable to the sediment sample types described above.

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 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method*

ISO 6059, *Water quality — Determination of the sum of calcium and magnesium — EDTA titrimetric method*

ISO 10523, *Water quality — Determination of pH*

3 Terms and definitions

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

3.1
artificial sediment
mixture of materials that mimic the physical components of natural sediment

Note 1 to entry: See 6.2.1.

3.2
control sediment
sediment (natural or artificial) that is used to assess the performance of the test organisms and test acceptability (i.e. clean)

Note 1 to entry: The results of control sediment testing are used for comparison to response of organisms in the contaminated test sediment(s) and for evaluating test validity.

Note 2 to entry: Used routinely to assess the acceptability of a test (6.2).

3.3
test sediment
discrete portion of sediment (field collected or spiked) to be tested for possible effects on amphipods due to contamination

3.4
spiked sediment
sediment to which a material has been added for testing

3.5
reference sediment
field collected near an area of concern with properties representing sediment conditions that closely match those of the sample(s) of the test sediment except for the degree of contamination

Note 1 to entry: It is often selected from a site uninfluenced by the source(s) of contamination but within the general vicinity of the sites where samples of test sediment are collected. Test results provide a site-specific basis for evaluating toxicity.

3.6
intermittent renewal
tests in which test solutions or overlying water are renewed during the test, based on deterioration of water quality

3.7
overlying water
water placed over the layer of sediment in the test container

Note 1 to entry: Also used to manipulate the sediment, if necessary (e.g. for preparing formulated sediment or mixtures of spiked sediment).

3.8
growth
increase in dry weight of test organisms during the experiment and expressed as mean dry weight per surviving amphipod

4 Principle

Young fresh water amphipods, *Hyalella azteca*, aged 2 d to 9 d and ranging in age by 1 d to 2 d, are exposed in groups of 10 organisms to a contaminated sediment or a test-chemical spiked sediment for 14 d and/or 28 d.[1] [2] [3] The end points for the test are percent mortality and growth inhibition assessed relative to organisms exposed concurrently to control sediment. The test is performed in glass containers with the ratio of sediment to water (volume:volume) being either 1:1,75 or 1:4 (e.g. 100 ml of sediment with 175 ml of overlying water or 100 ml of sediment with 400 ml of overlying water). Comparative testing of the two recommended sediment-to-water ratios showed no significant difference in test results using *Hyalella azteca*.[4] One advantage of the 1:4 sediment-to-water ratio is greater overlying water volume for chemical analysis. The exposure is primarily static unless renewal is triggered by deterioration of water in the control treatment (e.g. shifting pH affecting the form of background ammonia).

A long-term test option (i.e. 42 d) for whole sediment toxicity testing using *Hyalella azteca* is described in Annex E. End points of this long-term test include survival (Days 28, 35, and 42), growth (Days 28 and 42), and reproduction (number of young per female produced from Days 28 to 42).

A water-only method using *Hyalella azteca* is also described in Annex F. This method is a 14-day test of survival and growth using young amphipods exposed to samples of industrial or sewage effluents, fresh waters (e.g. receiving water), aqueous extracts, or chemical substances which are soluble or which can be maintained as stable suspensions or dispersions under the conditions of the test.

5 Test environment

5.1 Facilities

The test facility shall be well ventilated, isolated from physical disturbances, and free from dust and fumes. Tests shall be carried out in a temperature-controlled room or chamber that maintains a temperature of (23 ± 2) °C in the test containers.

5.2 Lighting

All test containers shall receive direct, overhead illumination that provides normal laboratory lighting (i.e. 100 lx to 1 000 lx) at the air/water interface. Illumination should be uniform and shall have a day/night cycle (photoperiod) of 16 h of daylight and 8 h of darkness.

6 Reagents, test organisms, and materials

Use only reagents of recognized analytical grade, unless otherwise specified.

6.1 Test organism

Hyalella azteca is an epibenthic sediment-burrowing detritivore that lives in close contact with the surficial 1 cm or 2 cm of fresh water sediments. They reside in temperate lakes, ponds, and slow-flowing streams and are widely distributed on the North and South American continents.[1] [2] [3] Young *Hyalella azteca* are obtained from laboratory cultures maintained under the conditions of temperature, photoperiod, and food identical to those in the test. The species identification should be confirmed by qualified personnel experienced in identifying fresh water amphipods using the distinguishing taxonomic features described in Annex A and in previous publications.[2] [5]

6.1.1 Life stage and size

Amphipods used for the test shall be between the ages of 2 d and 9 d and shall not vary in age by more than 2 d. A method for culturing *Hyalella azteca* and for obtaining known-age test organisms is provided in Annex B. If growth is expressed as mean size at the end of the test, a mean length of organisms should be determined at test initiation.

6.1.2 Source

All amphipods used in a test shall be derived from the same population and source. Sources of animals to be used to establish cultures include government or private laboratories which are culturing *Hyalella azteca* for sediment toxicity tests or a reputable biological supply company.[1] [2] [3] A list of possible sources of *Hyalella azteca* is provided in Annex C.

6.2 Control sediment

Each sediment toxicity test shall include a control with a minimum of five replicate test containers containing control sediment. Responses of organisms exposed to control sediment during a test provide measurements for determining test validity (see 10.3), evidence of the health and normal behaviour of

the test organisms, and a basis for interpreting data derived from the test sediments. Control sediment is either natural sediment or artificial (i.e. formulated) sediment.

6.2.1 Natural sediment

Natural sediment taken from a fresh water or slightly brackish (< 15 ‰) collection site removed from known sources of contaminants, and for which there is known control performance with *Hyalella azteca*, can be used as the control sediment for a test or as a clean material for spiking a test chemical. If sediment pore water has any measureable salinity, the testing laboratory shall follow a suitable acclimation procedure to ready adult *Hyalella azteca* for use as brood organisms and to ensure salinity-adapted young amphipods are used during testing.

6.2.2 Artificial sediment

The following artificial sediment can be used as a control for fresh water sediment tests or as a clean material for spiking a test chemical. This recipe is based on the artificial sediment recommended in ISO 10872.

Mix the following components thoroughly in the given proportions.

Al_2O_3:	20 %
$CaCO_3$:	1 %
Dolomite (clay):	0,5 %
Fe_2O_3:	4,5 %
Silica sand (mean particle size 0,063 mm):	30 %
Silica sand (0,1 mm to 0,4 mm):	40 %
Peat (decomposed peat from a raised bog, untreated, finely ground and < 1 mm sieved):	4 %

There are a number of acceptable approaches to preparing and conditioning artificial sediment. In general, the following attributes should be considered when selecting a formulation for a control or test sediment.

a) should support the survival, growth, or reproduction of a variety of benthic organisms;

b) should provide consistent acceptable biological end points for a variety of species;

c) should comprise standard constituents that are readily available to test laboratories;

d) should be free from concentrations of contaminants that might cause adverse effects to test organisms.

Acceptable artificial sediment options are outlined in Environment Canada guidance on this subject, including techniques for spiking test chemicals into sediment.[6]

6.3 Overlying water

6.3.1 Natural fresh water

Natural fresh water includes an uncontaminated supply of groundwater or surface water. If the objective of testing is to simulate field site conditions, natural water can be diluted with a high purity distilled or deionized water until a desired hardness is achieved. Water taken from the site where sediment is collected can also be used. Surface water should be filtered through a fine-mesh net (e.g. 30 µm) to remove potential predators or competitors. Dechlorinated water is not recommended as overlying water because its quality is often quite variable and it could contain unacceptably high concentrations of chlorine, chloramines, fluoride, copper, lead, zinc, or other contaminants.

6.3.2 Reconstituted water

If reconstituted fresh water is used as overlying water in *Hyalella azteca* tests, the following artificial medium shall be prepared in deionized water:[7]

$CaCl_2$	110,98 mg/l
$NaHCO_3$	84,01 mg/l
$MgSO_4$	30,09 mg/l
KCl	3,728 mg/l
NaBr	1,029 mg/l

The mixture is aerated for 24 h before use to adjust the dissolved oxygen (DO) and to stabilize pH. The concentrations of salts can be adjusted to be of similar composition to a receiving water of interest. However, the Ca:Br ratio shall be kept constant because these ions are essential for *Hyalella azteca* and must be present together.[8] Na^+ and HCO_3^- are the most essential ions for *Hyalella azteca* survival, and Mg^{2+} and K^+ are needed for optimal growth and reproduction.[7] [9] Due to lack of confirmed influence, ranges for alkalinity and hardness have not been defined. Standard use of the above reconstituted water recipe in culturing and as testing water has confirmed acceptable alkalinity and hardness levels for *Hyalella azteca*. Conductivity, pH, hardness, DO, and alkalinity are measured in each batch of reconstituted water. A given batch of reconstituted water shall not be used for longer than 4 weeks.

6.3.3 Dissolved oxygen

The DO content of the water overlying the sediment is ideally to be 90 % to 100 % of the air-saturation value at test initiation and throughout the test period. This level of DO is maintained by gentle aeration using filtered, oil-free compressed air. The rate of aeration should not suspend the sediment (e.g. 2 bubbles/s to 3 bubbles/s).

6.4 Food

There are two food options for use in the *Hyalella azteca* test. Both commercial fish food and an inoculum of a mixture of yeast, Cerophyll™[1)], and trout chow (YCT) have been proven suitable for *Hyalella azteca* under the defined test conditions.[1] [2] [3] [10] [11] [12]

There are also two options for the frequency of feeding. Test organisms are fed either once daily or three times weekly (on non-consecutive days) throughout the test. An identical food ration is added to each test chamber on each feeding occasion.[11] The ration provided shall be adequate to enable acceptable survival and growth of *Hyalella azteca* during the test period, but must not be excessive.

6.4.1 Option 1: Fish food

Commercial fish food flakes (e.g. Tetrafin™, Tetramin™, or Nutrafin™)[2)] can be used as a food source for test organisms during the test. Food can be ground and sieved so that flakes are uniform in size or can be prepared as a slurry by mixing the fish flakes with clean water. Fish food flakes should be stored at room temperature in a sealed container.

If daily feeding is chosen, 2,7 mg of fish flakes (dry weight) is added to each test container on the first day of the test (the day the amphipods are placed in the test containers), as well as once per day thereafter until the day the test ends. If the option of feeding three times per week is chosen, 6,3 mg of fish flakes

1) Cerophyll™ can be obtained from Ward's Scientific as "Cereal Grass Media - Cerophyll". This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

2) Tetrafin™, Tetramin™, and Nutrafin™ are examples of fish food flakes available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

(dry weight) is added three times per week (starting on the first day of the test) to each test chamber on non-consecutive days (e.g. on Mondays, Wednesdays, and Fridays), until the day the test ends. Both rations provide approximately the same overall feeding rate; however, daily feeding might be preferred because food is then always available.[6]

6.4.2 Option 2: Yeast/Cerophyll™[3]/trout chow (YCT)

A second food combination based on the U.S. Environmental Protection Agency (U.S. EPA) and Environment Canada test methods[1] [2] is also recommended.

The formula for preparing YCT is given in Annex D. If daily feeding is chosen, an inoculum of 1,5 ml (equivalent to 2,7 mg food, dry weight) of a mixture of yeast, Cerophyll™[3], and trout chow is added daily to each test chamber on the first day of the test (i.e. the day the amphipods are placed in the test containers), as well as once per day thereafter until the day the test ends. If the option of feeding three times per week is chosen, an inoculum of 3,5 ml YCT (equivalent to ~ 6,3 mg food, dry weight) is added three times per week (starting on the first day of the test) to each test chamber on non-consecutive days (e.g. on Mondays, Wednesdays, and Fridays), until the day the test ends. Both rations provide approximately the same overall feeding rate; however, daily feeding might be preferred because food is then always available.

YCT can be stored frozen. Thawed aliquots of unused YCT can be stored in darkness at (4 ± 3) °C but shall be discarded after 14 d.

6.5 Reference substance

Cadmium chloride ($CdCl_2$), copper sulphate ($CuSO_4$), sodium chloride (NaCl), and potassium chloride (KCl) are all acceptable reference substances.[1] [2] [3] [10] Material Safety Data Sheets (MSDS) for these substances should be consulted prior to use by laboratory personnel, as necessary.

7 Apparatus

Ordinary laboratory apparatus is used for organism culturing and testing.

7.1 Temperature-controlled room, chamber, or water bath.

The system chosen shall maintain a temperature of (23 ± 2) °C in the test containers.

7.2 Measuring apparatus.

Use the apparatus and/or instruments for measuring DO, pH, hardness, conductivity, alkalinity, ammonia, light intensity, and temperature as specified in ISO 10523, ISO 5814, and ISO 6059.

7.3 Test containers.

All containers and accessories such as sieves that might contact the organisms, control or test sediment, and overlying water during sorting, handling, and testing shall be made of non-toxic materials (e.g. glass, stainless steel, Nalgene™[4] nylon, porcelain, polyethylene, polypropylene, fibre-glass) cleaned and rinsed with distilled water, deionized water, dechlorinated laboratory water, reconstituted water, or natural water from an uncontaminated source. Materials such as copper, zinc, brass, galvanized metal, lead, and natural rubber shall not come in contact with this apparatus and equipment or with samples of control, reference or test sediment, overlying water, or test containers. Before initiating a test, ensure all test containers and associated labware are clean and free of all contaminants from previous use.

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3) Cerophyll™ can be obtained from Ward's Scientific as "Cereal Grass Media - Cerophyll". This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

4) Nalgene™ is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.