SLOVENSKI STANDARD
SIST ISO 10872:2011

01-junij-2011

Kakovost vode - Določevanje učinkov strupenosti vzorcev usedlin in tal na rast, plodnost in razmnoževanje Caenorhabditis elegans (Nematoda)

Water quality - Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of Caenorhabditis elegans (Nematoda)

Qualité de l'eau - Détermination de l'effet toxique d'échantillons de sédiment et de sol sur la croissance, la fertilité et la reproduction de Caenorhabditis elegans (nématodes)

Ta slovenski standard je istoveten z: **ISO 10872:2010**

ICS:

13.060.70	Preiskava bioloških lastnosti vode	Examination of biological properties of water

SIST ISO 10872:2011 **en**

INTERNATIONAL STANDARD

ISO 10872

First edition
2010-06-15

Water quality — Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of *Caenorhabditis elegans* (Nematoda)

Qualité de l'eau — Détermination de l'effet toxique d'échantillons de sédiment et de sol sur la croissance, la fertilité et la reproduction de Caenorhabditis elegans *(Nematodes)*

Reference number
ISO 10872:2010(E)

PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

COPYRIGHT PROTECTED DOCUMENT

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

Published in Switzerland

Contents

Page

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 10872 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 5, *Biological methods*.

Introduction

Nematodes are the most abundant and species-rich group of metazoans in sediments and soils[1][2] and play an important role in benthic and soil food webs[3][4]. Nematodes are endobenthic organisms that are found at various trophic levels due to the evolution of different feeding types (bacterivorous, algal feeder, omnivorous, predators).

The test organism *Caenorhabditis elegans* (Maupas, N2 *var*. Bristol) is a bacterivorous nematode that is found primarily in terrestrial soils but it also occurs in aquatic sediments of polysaprobial fresh-water systems[5][6]. *C. elegans* is a well-studied organism and very easy to cultivate[7].

The test is designed for measurement of the response to dissolved and particle-bound substances[8][9][10]. It applies to the testing of sediments, soils, waste, pore water, elutriates and aqueous extracts.

Water quality — Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction of *Caenorhabditis elegans* (Nematoda)

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 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 growth, fertility and reproduction of *Caenorhabditis elegans*. The method applies to contaminated whole fresh-water sediment (maximum salinity 5 ‰), soil and waste, as well as to pore water, elutriates and aqueous extracts that were obtained from contaminated sediment, soil and waste.

2 Normative references

The following referenced documents are indispensable for the application 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.

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

ISO 7027, *Water quality — Determination of turbidity*

ISO 10390, *Soil quality — Determination of pH*

ISO 10523, *Water quality — Determination of pH*

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

3 Terms and definitions

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

3.1
agar plate
Petri dish filled with NGM agar (5.8)

3.2
aqueous control
water that serves as negative control for tests in aqueous samples

3.3
artificial control sediment
defined artificial sediment (5.12)

3.4
bacterial stock culture
stock culture of food bacteria

3.5
blank replicate
additional replicate that contains no test organism, but is treated in the same way as the other replicates of a sample

3.6
control
treatment that serves as negative control to which the effect in the respective test material is compared (3.2, 3.3, 3.7)

3.7
control soil
defined standard soil (5.13)

3.8
dauer larva
developmental stage adopted by *C. elegans* to endure periods of lack of food

NOTE Dauer larvae continue normal development if food is supplied.

3.9
exposed test organisms
individuals of *C. elegans* that are introduced at the beginning of the test

3.10
food medium
defined aqueous bacterial suspension (10.1)

3.11
J_1 stage
first of four juvenile stages (J_1 to J_4) in the development of *C. elegans*

3.12
overnight culture
defined culture of *Escherichia coli* in LB-medium (9.1.2)

3.13
starved plate
agar plate with dauer larvae

3.14
test material
discrete portion of a contaminated environmental sample (10.2) or solution of the reference substance (Clause 7)

4 Principle

Juvenile organisms of the species *C. elegans* are exposed to the environmental sample over a period of 96 h. In the controls, the exposed test organisms are able to complete a whole life cycle within this period. A toxic effect of an environmental sample occurs if the inhibition of growth, fertility or reproduction of *C. elegans* in comparison to a control (aqueous control, control sediment or soil) exceeds a certain threshold value. Toxicity can by quantified by the intensity of the effect as percentage inhibition.

5 Reagents

Use only reagents of recognized analytical grade.

5.1 Water, distilled or deionized water or water of equivalent purity, conductivity $\leqslant$ 10 µS/cm.

5.2 LB-medium.

Dissolve

— 0,5 g of casein peptone;

— 0,25 g of yeast extract;

— 0,5 g of sodium chloride (NaCl);

in 50 ml water in a 250 ml flask and autoclave for 20 min at 121 °C.

5.3 Cholesterol stock solution.

Dissolve 500 mg of powdered cholesterol in 100 ml of absolute ethanol (> 99 % purity) by stirring and gentle heating (< 50 °C). Replace ethanol lost through evaporation with ethanol.

5.4 Calcium chloride stock solution, 1 mol/l $CaCl_2$.

Dissolve 147 g of $CaCl_2 \cdot 2H_2O$ in 1 000 ml water and autoclave for 20 min at 121 °C.

5.5 Magnesium sulfate stock solution, 1 mol/l $MgSO_4$.

Dissolve 247 g of $MgSO_4 \cdot 7H_2O$ in 1 000 ml water and autoclave for 20 min at 121 °C.

5.6 Potassium hydroxide, KOH, pellets.

5.7 Potassium phosphate buffer, 1 mol/l KH_2PO_4.

Dissolve 136 g of KH_2PO_4 in 1 000 ml of water, adjust with KOH (5.6) to pH 6,0 $\pm$ 0,2, and autoclave for 20 min at 121 °C.

5.8 Nematode growth-medium agar (NGM agar).

Dissolve

— 2,5 g of casein peptone;

— 17 g of bacteriological agar;

— 3 g of NaCl;