

INTERNATIONAL STANDARD

ISO 16198

First edition
2015-01-15

Soil quality — Plant-based test to assess the environmental bioavailability of trace elements to plants

*Qualité du sol — Test végétal pour l'évaluation de la biodisponibilité
environnementale des éléments traces pour les végétaux*



Reference number
ISO 16198:2015(E)

© ISO 2015

ISO 16198:2015(E)



COPYRIGHT PROTECTED DOCUMENT

© ISO 2015

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

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		iv
Introduction		v
1 Scope		1
2 Normative references		1
3 Terms and definitions		2
4 Principle		3
5 Laboratory apparatus		5
6 Reagents		5
6.1 General.....		5
7 Biological and growing apparatus		6
7.1 Plant species.....		6
7.2 Biotest apparatus.....		6
7.3 Composition of the nutrient solutions.....		9
7.4 Climatic conditions in the growth chamber.....		10
8 Pre-treatment and analysis of soil or soil material sample		10
8.1 Sample size and particle size reduction.....		10
8.2 Analyses.....		10
9 Experimental and analytical procedure		11
9.1 Overview of the procedure.....		11
9.2 Selection and preparation of seeds.....		11
9.3 Preculture period: Germination and pre-growth in hydroponics.....		11
9.4 Preparation and incubation of soil or soil material.....		12
9.5 Test culture period: Plant growth in contact with soil or soil material.....		12
9.6 Plant harvests.....		13
9.7 Grinding and digestion of shoots and roots.....		13
9.8 Analytical determination.....		14
10 Validity criteria		14
11 Assessment of the results		14
11.1 Determination of trace element concentrations and uptake flux in plants.....		14
11.2 Data presentation.....		16
11.3 Expression of the results.....		16
12 Statistical analysis		16
12.1 General.....		16
12.2 Plant biomasses.....		16
12.3 Bioavailability end points.....		17
13 Test report		17
Annex A (informative) Plant species adapted to the biotest procedure		19
Annex B (informative) Technical drawings of the different components of the biotest		21
Annex C (informative) Seed selection and seed density in plant pot for a range of species tested with the standardized experimental procedure		24
Annex D (informative) Digestion and analysis of plant samples		26
Annex E (informative) Range of biomasses and trace element quantities in control plant pots		28
Annex F (informative) Ring-test		29
Bibliography		43

ISO 16198:2015(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 (see 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 (see 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.

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 190, *Soil quality*, Subcommittee SC 7, *Soil and site assessment*.

Introduction

One of the main objectives of ISO 17402 is to define a conceptual framework of the bioavailability of contaminants in soils and soil materials, and to provide a guidance for the selection of methods able to be standardized for the measurement of bioavailability. Bioavailability was thus defined according to three successive steps:

- a) “environmental availability”;
- b) “environmental bioavailability”;
- c) “toxicological bioavailability”.

The environmental bioavailability is consequently a prerequisite to the assessment of the toxicological bioavailability and is directly related to the impact of pollutants on major functions of soil in the ecosystem and more particularly to habitat and retention functions.

Environmental bioavailability can be estimated with either chemical or biological methods. In the case of trace elements, chemical methods are usually the cheapest, easy to perform, and some of them are already standardized at national or international level (e.g. ISO 19730). However, chemical methods which, strictly speaking, measure the environmental availability in soils have to be correlated with biological measurements before being used as indicators of environmental bioavailability. Whatever chemical methods are employed, none are designed per se to address the diversity of responses observed among different plant species or cultivars which can be attributed to a) the uptake behaviour of plants (i.e. sensitive, tolerant, accumulator, or hyper-accumulator of trace elements) and/or b) the ability of plants to alter the biological, physical and physical-chemical properties of their “bio-influenced zone” at the soil-root interface, i.e. the so-called rhizosphere. It could alternatively, be suggested to apply chemical methods directly to the rhizosphere but the sampling of the rhizosphere is definitely too tedious to be applied routinely.

For biological methods, four standardized biotests account for rhizosphere processes as they are based on soil-grown plants (ISO 11269-1, ISO 11269-2, ISO 17126, and ISO 22030). However, these were only designed to predict trace element phytotoxicity, i.e. the toxicological bioavailability. In these biotests, roots grow directly in the soil, therefore requiring a tedious washing procedure to reliably measure trace elements accumulated in the roots. Indeed, the amount of trace elements accumulated in shoots of non-accumulator plant species is not sufficiently sensitive to be used for assessing the environmental bioavailability of trace elements compared to the amount accumulated in the whole plant, roots included. Thus, there is still a need to develop biological methods accounting for rhizosphere processes and enabling to include the root compartment in order to properly estimate the environmental bioavailability of trace elements to plants.

Consequently, the present International Standard introduces a biotest based on the growing of roots in contact with the soil but without penetrating it. Although this experimental design is partly artificial, it enables a fair comparison of the bioavailability of trace elements between tested soils. In addition, the end point measured can be more directly related to the measurement of the environmental availability than any end point based on the measurement of toxicity.

Soil quality — Plant-based test to assess the environmental bioavailability of trace elements to plants

1 Scope

This International Standard specifies the plant-based test, hereafter called the biotest. It enables estimation of the environmental bioavailability of trace elements to plants either basically as the concentration in shoots and roots or in a more integrative way as the net uptake flux in plants. The biotest procedure includes two successive steps: (i) a pre-growth of plants in hydroponics and (ii) a growth of plants in contact with soil samples. The concentration in shoots and roots as well as the net uptake flux of trace elements in plants are determined at the end of the second step of the biotest procedure.

This biotest is applicable to the assessment of environmental bioavailability of trace elements to plants, more particularly to agricultural plants, in soils or soil materials under oxic conditions, considering that

- three plant species (cabbage, *Brassica oleracea*; tall fescue, *Festuca arundinacea*; tomato, *Lycopersicon esculentum*; 7.1) are suggested in the standardized biotest procedure, but additional target-plant species can also be used (see 7.1, Annex A), and
- the standardized biotest procedure is validated for a range of trace elements including arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn), but additional trace elements can also be accounted for (see Annex A).

The biotest can be applied to soils and soil materials, including soils amended before or after field sampling with composts, sludges, wastewaters, and other (waste) materials.

NOTE 1 This biotest is not designed to assess the environmental bioavailability of trace elements that are prone to volatilisation or resulting from uptake occurring in plant leaves following, e.g. atmospheric fallout.

NOTE 2 This biotest is not designed to assess the environmental bioavailability to plants of organic contaminants. A similar experimental procedure could be used but the physical separation between plant roots and soil using a polyamide mesh needs to be adapted to avoid organic contaminant sorption on the mesh.

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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11269-2, *Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early growth of higher plants*

ISO 11277, *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation*

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

ISO 16198:2015(E)

3 Terms and definitions

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

3.1 contaminant

substance or agent present in the soil as a result of human activity

[SOURCE: ISO 11074:2005, 3.5.1]

Note 1 to entry: There is no assumption in this definition that harm results from the presence of the contaminant

3.2 environmental availability

fraction of contaminant physico-chemically driven by desorption processes potentially available to organisms

[SOURCE: ISO 17402:2008, 3.4]

3.3 environmental bioavailability

fraction of the environmentally available compound which an organism takes up through physiologically driven processes

[SOURCE: ISO 17402:2008, 3.5]

3.4 habitat function

ability of soil/soil materials to serve as a habitat for micro-organisms, plants, soil-living animals, and their interactions (biocenosis)

[SOURCE: ISO 11074:2005, 3.4.3]

3.5 trace element

chemical element in soil occurring at concentration generally less than 100 mg kg⁻¹

Note 1 to entry: Given according to Reference [16].

3.6 retention function

ability of soil/soil materials to adsorb pollutants in such a way that they cannot be mobilized via the water pathway and translocated into the terrestrial food chain

[SOURCE: ISO 11074:2005, 3.4.13]

3.7 rhizosphere

volume of soil around living roots that is influenced by root activities

Note 1 to entry: Given according to Reference [17].

3.8 soil

upper layer of the earth's crust transformed by weathering and physical/chemical and biological processes. It is composed of mineral particles, organic matter, water, air, and living organisms organized in genetic soil horizons

[SOURCE: ISO 11074:2005, 2.1.8]

3.9

soil material

material coming from soil and displaced and/or modified by human activity, including excavated soil, dredged materials, manufactured soils, and treated soils and fill materials

[SOURCE: ISO 17402:2008, 3.16]

3.10

toxicological bioavailability

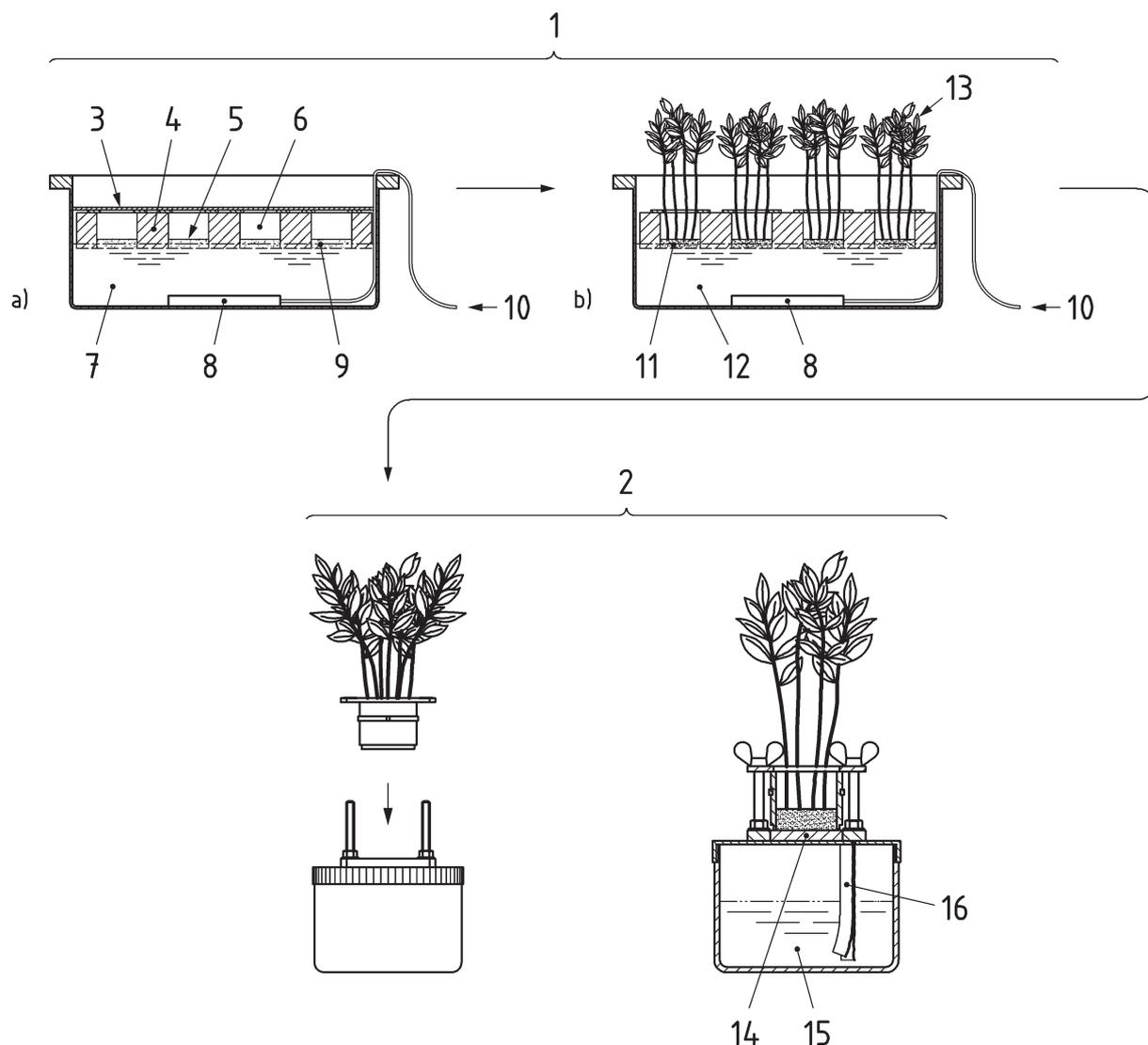
internal concentration of pollutant accumulated and/or related to a toxic effect

[SOURCE: ISO 17402:2008, 3.18]

4 Principle

This International Standard describes the experimental procedure of the biotest developed initially by References [18], [19], and [20]. This biotest consists of two successive steps of plant growth (see [Figure 1](#)). During the first step (i.e. preculture period), plant seedlings are grown in hydroponics for 14 d to achieve an adequate plant biomass and a dense, planar root mat. During the second step (i.e. test culture period), the root mat of pre-grown plants is put in contact for 8 d with a 6 mm-thick layer of soil sample sieved to 2 mm.

ISO 16198:2015(E)



Key

- | | | | |
|---|--|----|-------------------------|
| a | seed germination (7 d) | 8 | air diffuser |
| b | seedling pre-growth (7 d) | 9 | 30-µm mesh |
| 1 | preculture period in hydroponics - 14 d | 10 | air |
| 2 | test culture period soil-plant contact - 8 d | 11 | root mat |
| 3 | aluminium foil | 12 | nutrient solution 2 |
| 4 | floating platform | 13 | shoots |
| 5 | seeds | 14 | soil layer (6 mm thick) |
| 6 | plant pot | 15 | nutrient solution 3 |
| 7 | nutrient solution 1 | 16 | filter paper wicks |

Figure 1 — The two-step procedure of the biotest

A set of control plants is harvested at the end of the preculture period in hydroponics to determine the pools of trace elements in plant shoots and roots before exposure to soil. Whole plants (shoots and roots) are then harvested at the end of the test culture period. Biomasses and trace element concentrations in shoots and roots are determined. The end points of the biotest are a) the concentration of trace elements in shoots and roots at the end of the test culture period and b) the net uptake flux of trace elements in the whole plants during the test culture period. If these end points are usually correlated,^[21] the uptake flux is expected to be more representative of the trace element bioavailability to plants during the test

culture period (i.e. the exposure to tested soils) as, conversely to concentrations, the uptake flux does not include the portion of trace elements taken up during the preculture period (11.1).

As plant growth during the pre-culture period is usually sufficient to prevent the occurrence of phytotoxic symptoms induced by adverse soil chemical properties or excessive accumulation of trace elements in plant, the biotest enables a fair comparison of trace element bioavailability over a broad range of soils, including heavily contaminated soils.

5 Laboratory apparatus

The following equipment shall be used. All equipment that comes into contact with the sample (soils, plants, or reagents) shall not adsorb substantially trace elements and shall not contaminate the sample.

- 5.1 **Sieving equipment**, nominal screen size 2 mm.
- 5.2 **Crushing equipment**, jaw crusher and cutting device.
- 5.3 **Balance**, with an accuracy of at least 100 mg.
- 5.4 **Balance**, with an accuracy of at least 1 mg.
- 5.5 **Growth chamber**, suitable for maintaining specific climatic conditions as specified in 7.4.
- 5.6 **Ventilated oven**, suitable for drying soil or soil material at 25 °C and shoots and roots at 50 °C.
- 5.7 **Scissors**, with blades made of zirconium oxide.
- 5.8 **Grinder and marbles**, made of zirconium oxide.

6 Reagents

6.1 General

Use reagents of analytical grade purity with a concentration of investigated trace elements (e. g. As, Cd, Co, Cr, Cu, Ni, Pb, and Zn) lower or equal to 5 mg kg⁻¹. Water used shall comply with grade 3 according to ISO 3696.

- 6.2 **Water**, distilled or demineralized with a specific conductivity of at most 5 µS cm⁻¹ at 25 °C and a pH within the range 5,0 to 7,5.
- 6.3 **Hydrogen peroxide** (H₂O₂, 34,01 g mol⁻¹).
- 6.4 **Calcium chloride dihydrate** (CaCl₂·2H₂O, 147,07 g mol⁻¹).
- 6.5 **Boric acid** (H₃BO₃, 61,83 g mol⁻¹).
- 6.6 **Calcium nitrate tetrahydrate** (Ca(NO₃)₂·4H₂O, 236,15 g mol⁻¹).
- 6.7 **Potassium nitrate** (KNO₃, 101,1 g mol⁻¹).
- 6.8 **Magnesium sulfate heptahydrate** (MgSO₄·7H₂O, 246,48 g mol⁻¹).