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

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English Version

Soil quality - Plant-based test to assess the environmental bioavailability of trace elements to plants (ISO 16198:2015)

Qualité du sol - Test végétal pour l'évaluation de la biodisponibilité environnementale des éléments traces pour les végétaux (ISO 16198:2015)

Bodenbeschaffenheit - Pflanzenbasierter Test zur Beurteilung der umweltrelevanten Bioverfügbarkeit von Spurenelementen für Pflanzen (ISO 16198:2015)

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Foreword

This document (EN ISO 16198:2015) has been prepared by Technical Committee ISO/TC 190 “Soil quality” in collaboration with Technical Committee CEN/TC 345 “Characterization of soils” the secretariat of which is held by NEN.

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Full document details: BS EN ISO 16198:2015

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**

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Foreword

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The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 7, *Soil and site assessment*.

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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.

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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*

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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]

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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.

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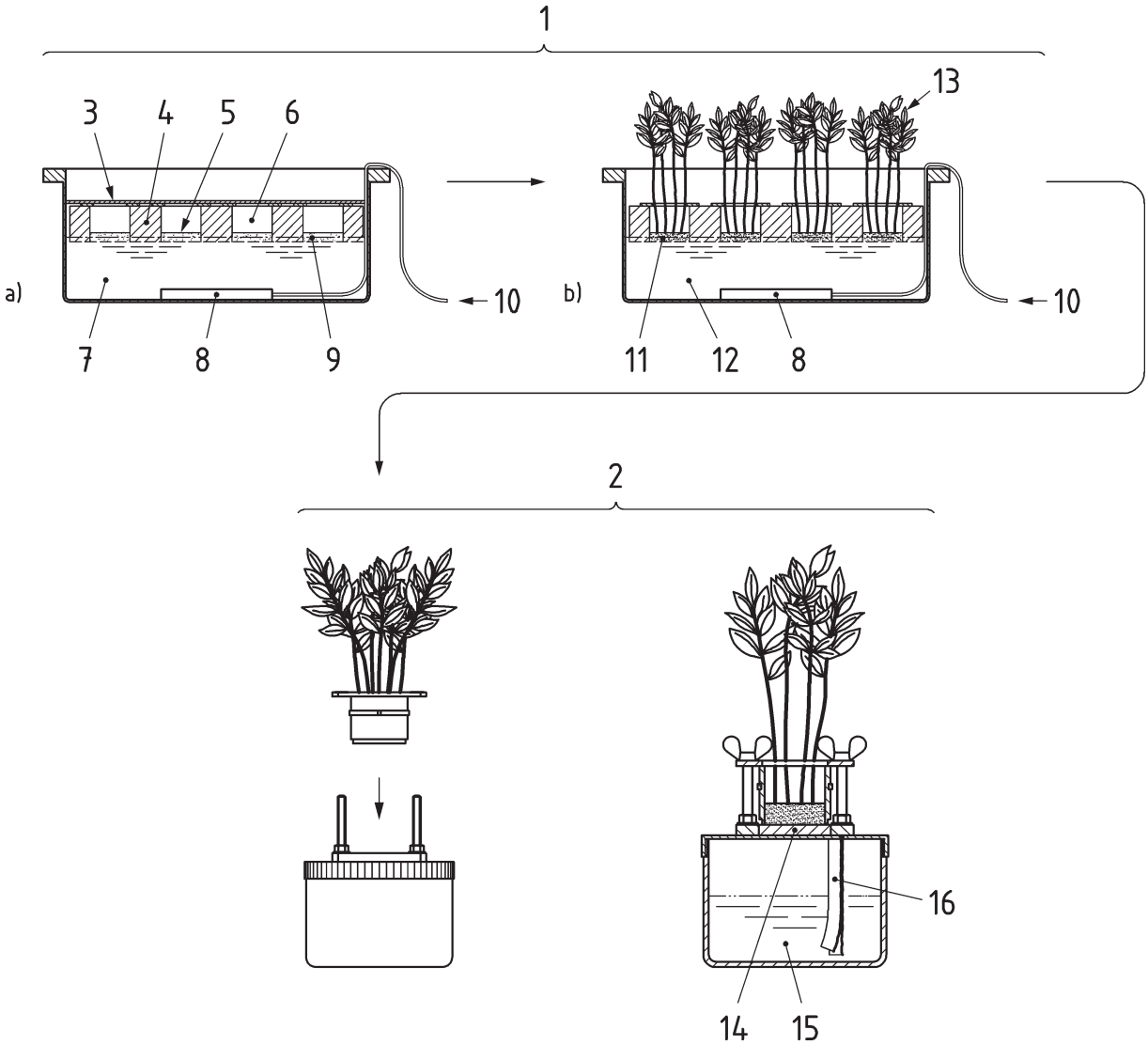

Key

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

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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 (H_2O_2, 34,01 g mol^{-1}).

6.4 Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, 147,07 g mol^{-1}).

6.5 Boric acid (H_3BO_3, 61,83 g mol^{-1}).

6.6 Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, 236,15 g mol^{-1}).

6.7 Potassium nitrate (KNO_3, 101,1 g mol^{-1}).

6.8 Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$, 246,48 g mol^{-1}).

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6.9 Potassium phosphate (KH_2PO_4, 136,09 g mol^{-1}).

6.10 Ethylenediaminetetraacetic acid iron(III) sodium (NaFe(III)EDTA, 367,05 g mol^{-1}).

6.11 Copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$, 170,48 g mol^{-1}).

6.12 Manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$, 197,91 g mol^{-1}).

6.13 Zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$, 287,54 g mol^{-1}).

6.14 Sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$, 241,95 g mol^{-1}).

7 Biological and growing apparatus

7.1 Plant species

The three following species, i.e. cabbage (*B. oleracea*), tall fescue (*F. arundinacea*), and tomato (*L. esculentum*), are used during the biotest deployment. These three plant species were selected among non-accumulator, common agricultural species for their ability to collectively maximize the phytoavailability of trace elements (more specifically that of As, Cd, Cu, Pb, and Zn) in soils exhibiting a broad range of physical-chemical properties and origin of trace element.[21] For each of the three species, the following cultivars are recommended: castelard for *B. oleracea*, calina for *F. arundinacea*, and fline for *L. esculentum*. However, in the only case where recommended cultivars are not commercially available other cultivars may be used provided that they exhibit an adequate growth and homogeneous root mat during the biotest procedure for the different soils tested. Specify in the test report the reasons for selecting different cultivars than those recommended. For a given cultivar, seeds used for a single set of experiment shall come from the same batch.

Additional species can be selected, e.g. species with specific physiological characteristics or with ecological, agricultural or economic significance in certain regions of the world or for specific site assessment, provided that these species exhibit an adequate growth and homogeneous root mat during the biotest procedure for the different soils tested. A list of plant species adapted to be grown in the biotest, but only partly validated for the standardized procedure, is given in Annex A. Specify in the test report the reasons for selecting additional species.

NOTE 1 The biotest procedure should be used with untreated seeds (i.e. not treated with any pesticide) as much as possible. If not feasible, specify it in the test report.

NOTE 2 If other cultivars than those recommended are used, it is to note that this can alter the biotest measurement in a similar extent than if different species were used.

7.2 Biotest apparatus

The following apparatus shall be used. Apparatus that comes into contact with the sample (soils, plants or reagents) shall not adsorb the component of interest and shall not contaminate the sample.

The plant-receiving pot (i.e. plant pot) is designed to contain the whole plants from the beginning of the preculture period to the end of the test culture period. The plant pot enables plants to develop a planar and dense root mat while maintaining a physical separation with the tested soil sample. The plant pot consists in a cylinder fitted to an upper plate at the top and closed at the bottom with a polyamide mesh (30 µm pore size) using an adjustable clamp (see Figure 2). The mesh shall be well tightened.

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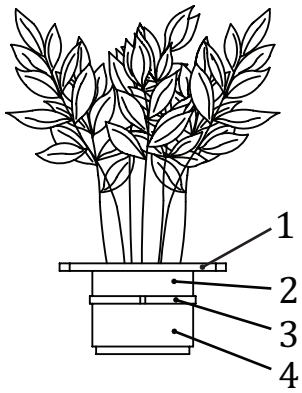

Key

1 upper plate

2 cylinder

3 adjustable clamp

4 30 µm polyamide mesh

Figure 2 — Plant-receiving pot assembly

The assembly used for the first step, i.e. the pre-culture period, is designed to enable seeds to germinate and for seedlings to develop a dense and planar mat of roots in hydroponics. This assembly enables a close contact between seeds or seedling roots and the nutrient solution. This assembly consists in a perforated platform floating over a tank-containing nutrient solution. Perforations passing through the floating platform enable to lodge plant pots. The nutrient solution is continuously aerated with a bubbling system composed of an air-pump, capillary tubes connected with derivations and diffusers diving into the nutrient solution (see Figure 3). The floatability of the platform is critical to ensure a homogeneous contact of all the plant pots in a tank with the aerated nutrient solution. This assembly also limits the exposure of the nutrient solution to light radiations, thereby avoiding algal development.

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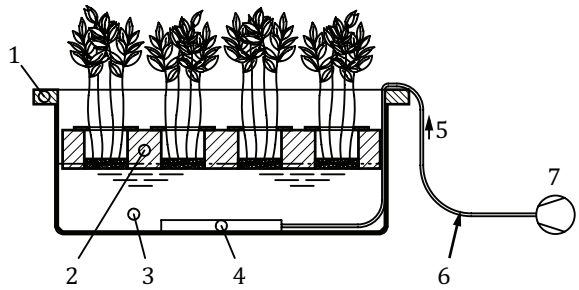
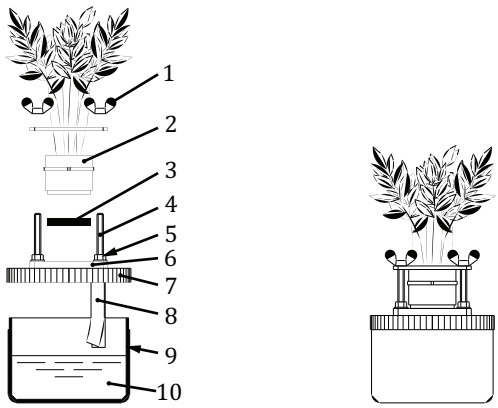


Key

1 tank
2 perforated floating platform
3 nutrient solution 1 or 2
4 air diffuser
5 air
6 tube
7 air-pump

Figure 3 — Assembly used for the preculture period

The assembly used for the second step, i.e. the test culture period, is designed to enable a close contact between the root mat and the soil layer. It is made of two parts and three filter paper wicks sandwiched in between: a) a contact assembly that firmly press the plant pot over the soil layer using fastenings and b) a 0,5 dm^3 screw-top jar filled with the nutrient solution 3 (see Figure 4). This assembly enables (i) the root mat to be maintained in contact with the whole surface area of the soil layer, and (ii) the filter paper wicks to remain fully moistened during the entire duration of the test culture period.



Key

1 wing nut
2 plant pot
3 soil layer (6 mm thick)
4 screw
5 screw nut
6 soil-receiving plate
7 screw-top
8 filter paper wicks
9 screw-top jar
10 nutrient solution 3

Figure 4 — Soil-plant contact assembly used for the test culture period

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Except for the adjustable clamp, the filter paper wicks and the polyamide mesh, all the components of the plant-based test are reusable provided that they are subjected to a two-step washing, firstly, in hot water to remove adhering mucilage and microbial biofilms then secondly, in a volume fraction of 10 % HNO_3, followed by a thorough rinsing with distilled or demineralized water. A thorough list of the components along with specification is given in Table 1 for information and technical drawings are given in Annex B. Home-made apparatus may be built provided that the size of the different parts remains proportional and that component specification is similar.

Table 1 — Components of the biotest (informative)

Component		No.[a]	Quantity[b]	Specification
Plant pot	Cylinder	1	1	PVC, high density, high temperature, for food contact
	Upper plate	2	1	HDPE for food contact
	Polyamide mesh	/	1	100 × 100 mm, 30 µm pore size
	Adjustable clamp	/	1	180 mm × 2,4 mm
Preculture period	Tank[c]	3	1	12 dm^3, 400 mm×294 mm × 165 mm, Opaque HDPE for food contact
	Perforated floating platform	4	1	Extruded polystyrene platform
	Two outputs Air-pump	/	1	Air flow 100 - 200 $dm^3\ h^{-1}$ output^{-1}
	Capillary tube	/	2	PVC, int. diam. ca. 4 mm
	Derivation	/	1	If necessary
	Air-diffuser	/	2	Ceramic diffuser, 100 × 10 mm
Test culture period	Screwtop jar	5	1	Opaque and white PP for food contact
	Filter paper wick	/	3	Hardened ashless filter paper
	Soil-receiving plate	6	1	HDPE for food contact, 6 mm thickness
	Screw	/	4[d]	HDPE
	Screw nut	/	8[d]	HDPE
	Wing nut	/	4[d]	HDPE

[a] Component number as referenced in the technical drawings depicted in Annex B.

[b] For one item of each of the three biotest components (i.e. plant pot and apparatus for preculture and test culture periods).

[c] Adapted for 12 plant pots per tank filled with 6 dm^3 of nutrient solution.

[d] Only two screws can be used to reduce time-fitting, with only four screw nuts and two wing nuts.

7.3 Composition of the nutrient solutions

Three different nutrient solutions shall be prepared for the deployment of the biotest for (i) seed germination, (ii) seedling pre-growth in hydroponics [steps (i) and (ii) are included in the preculture period], and (iii) plant growth during the test culture period.

During seed germination (preculture period), the nutrient solution 1 shall be composed of 600 $\mu mol \cdot dm^{-3}$ $CaCl_2$ (6.4) and 2 $\mu mol \cdot dm^{-3}$ H_3BO_3 (6.5).

During seedling growth in hydroponics (preculture period), the nutrient solution 2 shall be prepared by adding the nutrients in the following order and concentration: 500 $\mu mol \cdot dm^{-3}$ KH_2PO_4 (6.9); 2 000 $\mu mol \cdot dm^{-3}$ KNO_3 (6.7); 2 000 $\mu mol \cdot dm^{-3}$ $Ca(NO_3)_2$ (6.6); 1 000 $\mu mol \cdot dm^{-3}$ $MgSO_4$ (6.8);

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