

## **Soils, sludges, pretreated biowastes — Main element — Complementary element**

*Boden, Schlamm, behandelte Bioabfälle — Haupt-Element — Ergänzendes Element*

*Sols, boues, bio-déchets prétraités — Élément central — Élément complémentaire*

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## Foreword

This document TC xxx WI zzz has been prepared by Technical Committee CEN/TC xxx “”, the secretariat of which is held by yyy.

This document is a working document.

The following TC's have been involved in the preparation of the standard:

This standard is applicable and validated for several types of matrices. The table below indicates which ones.

[table to be filled and amended by the standards writer]

Material	Validated	Document
Waste	<input type="checkbox"/>	[reference]
Sludge	<input type="checkbox"/>	
Soil	<input type="checkbox"/>	

## Introduction

Safety warning

### 1 Scope

This Standard specifies a test procedure for the assessment of contamination by germinating plant seeds and propagules on soil, treated biowaste and sludge.

### 2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

EN 13037      Soil improvers and growing media – Determination of pH

EN 13038      Soil improvers and growing media – Determination of electrical conductivity

EN 13040      Soil improvers and growing media - Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory compacted bulk density

### 3 Terms and definitions

For the purposes of this European Standard, the following term and definition apply.

#### 3.1

##### **test sample**

Material after sample preparation due to clause 7

### 4 Principle

The development of plants, whether from seed or other plant propagules, is determined after a 28 days incubation period under controlled conditions.

### 5 Reagents

**5.1** Water of class 2 (tap water) according to EN ISO 3696

**5.2** raised bog peat (H3 – H5, according to von Post scale), free of viable seeds and plant propagules

**5.3** Solution of Gibberellic acid (C<sub>19</sub>H<sub>22</sub>O<sub>6</sub>), 1g · L<sup>-1</sup>

**5.4** Calcium carbonate, CaCO<sub>3</sub>

**5.5** Seeds of cress (*Lepidium sativum* ssp. *sativum*) or Chinese cabbage (*Brassica campestris*, var. *chinensis*), germination capacity > 90%, seeds of barley (*Hordeum vulgare*), germination capacity > 90%

### 6 Apparatus

**6.1** Seed tray, height ≥ 50 mm, with bottom perforation (e.g. plastic tray, 430 mm x 330 mm x 60 mm)

**6.2** Capillary mat: to be specified

**6.3** Perforated plastic sheet: to be specified

**6.4** Thin fleece for covering the trays to avoid air born seed contamination: to be specified

**6.5** Testing facility with temperature monitoring (monitoring range between 18°C and 26°C) and a lighting intensity of at least 10 W · m<sup>-2</sup> or 2000 lux for 12 hours, e.g. greenhouse, plant growth room

## **7 Preparation of the sample**

### **7.1 Treated Biowaste**

#### **7.1.1 General preparation**

The sample preparation has to be carried out in accordance with EN 13040, clause 8. For this test, material < 20 mm is used deviating from EN13040 in that all the test sample has to pass through the sieve, even if the retained volume fraction is greater than 10%. The fraction obtained after sieving must have a volume equal or greater than 3 litres.

#### **7.1.2 Dilution of the test material**

One part of the sample material is thoroughly mixed with 2 parts of peat (volume/volume) to get the test sample. The pH of the test sample according to EN 13037 has to be in the range between 5.5 and 7.0. If the value is < 5.5, it has to be raised by using calcium carbonate (5.5). If the value is above this range, the material has to be further diluted with peat (5.2) until the desired range is reached. If the electrical conductivity of the test sample according to CEN 13038 is > 50 mS · m<sup>-1</sup>, the sample has to be further diluted using peat (5.2) until the electrical conductivity does not exceed 50 mS · m<sup>-1</sup>. For assessing the proper dilution ratio, the electrical conductivity of the mixing component can be assumed to be between 1 and 4 mS · m<sup>-1</sup>.

### **7.2 Soil**

Soil is sieved < 5mm with only stones remaining in the retained fraction. The fraction obtained after sieving must have a volume equal or greater than 7 litres. The pH (water) of the test sample according to ISO 10390 has to be in the range between 5.5 and 7.0. If the value is < 5.5, it has to be raised by using calcium carbonate (5.5). If the value is above this range, the material has to be diluted with peat (5.2) until the desired range is reached. If the electrical conductivity according to CEN 13038 is > 50 mS · m<sup>-1</sup>, the sample has to be diluted using peat (5.2) until the electrical conductivity does not exceed 50 mS · m<sup>-1</sup>. For assessing of the dilution ratio, the electrical conductivity of the mixing component can be assumed to be between 1 and 4 mS · m<sup>-1</sup>.

### **7.3 Sludge**

At least 2000 ml of sample are mixed with 10000 ml peat to get the test sample. The pH of the test sample according to EN 13037 has to be in the range between 5.5 and 7.0. If the value is < 5.5, it has to be raised by using calcium carbonate (5.5). If the value is above this range, the material has to be further diluted with peat (5.2). If the electrical conductivity according to CEN 13038 is > 50 mS · m<sup>-1</sup>, the sample has to be diluted using peat (5.2) until the electrical conductivity does not exceed 50 mS · m<sup>-1</sup>. For assessing of the dilution ratio, the electrical conductivity of the mixing component can be assumed to be between 1 and 4 mS · m<sup>-1</sup>.

## **8 Procedure**

### **8.1 Experimental design**

In one replicate, a minimum volume of 1 litre solid material (according to EN 13040) or 200 ml (sludges) of the original material has to be tested. Diluted samples have to be thoroughly mixed and distributed to a respective number of seed trays. The test has to be performed in triplicate.

The bottom of the perforated seed tray (6.1) is covered by capillary mat (6.2) and a perforated plastic sheet (6.3). The test sample is filled into the tray and gently compressed to reach a layer thickness of approx. 20 mm. Then, the test material is saturated with a solution of 1g · L<sup>-1</sup> gibberellic acid (5.3), either by watering or subirrigation. If using subirrigation, the tray has to be immersed in the solution for 4 hours in the dark. Afterwards the tray is kept with free drainage until excess solution has dripped off.

The tray is kept in the testing facility (6.5) at a temperature suitable for plant germination (range between 18°C and 26°C) without exposure to direct sunlight for 28 days. During the whole testing period, the sample has to be kept moist by watering or subirrigation in intervals dependent on plant growth and environmental conditions in a practice related manner (good horticultural practice). In order to reduce desiccation and to avoid air-born

seed contamination, the trays are covered with a thin fleece (6.4) as shown in Fig. 1. The germinated plants have to be counted and removed (except if identification is asked) once a week.

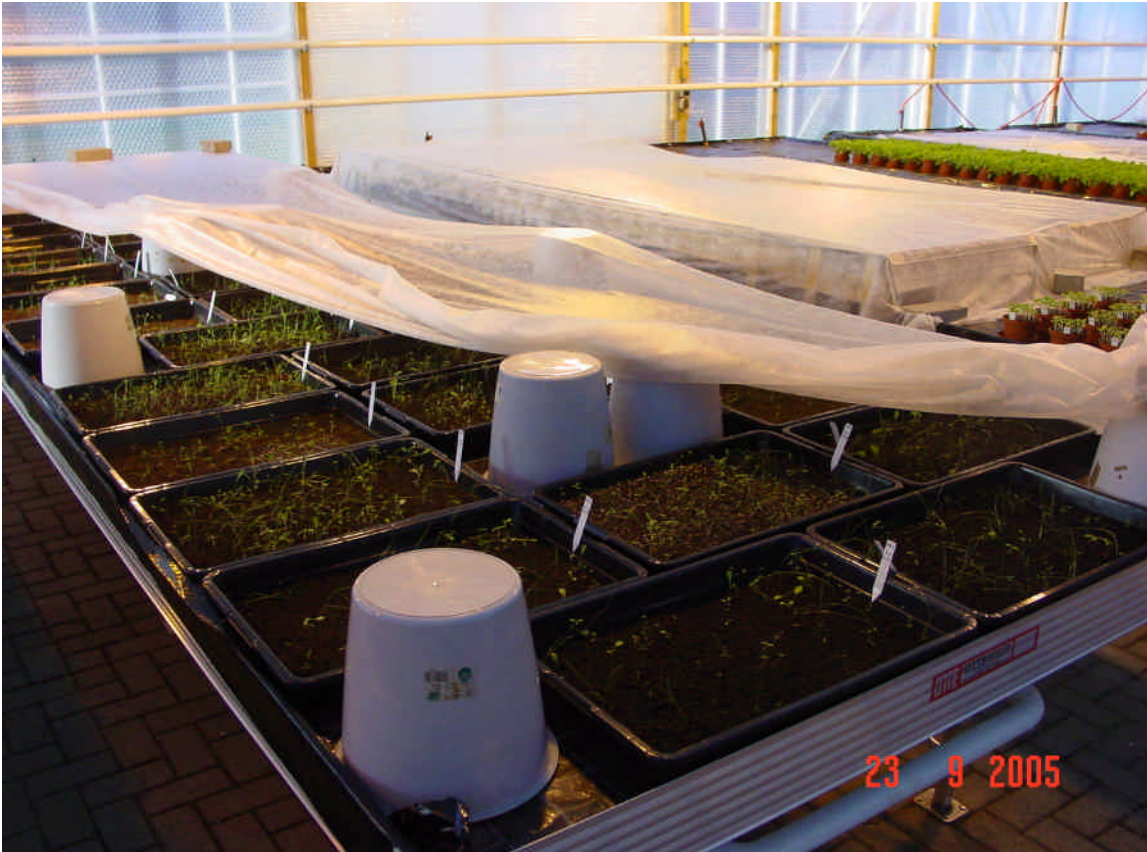


Fig. 1: Cover of the seed trays using a thin fleece

## 8.2 Validation of the test

To assess the influence of the environmental conditions, the germination of cress or chinese cabbage and barley in the test substrate is monitored. After preparing the test sample as described in clause 7 and the filling of the trays (clause 8.1), 15 seeds per litre substrate of cress or chinese cabbage and barley, respectively, are evenly distributed on the surface and covered with a thin layer of the test sample (control sample). Afterwards, the test is continued as described in clause 8.1. The control is performed in triplicate. If the germination rate of cress/chinese cabbage and barley in the control sample is less than 90%, the results of the test are not valid.

*Remark:*

*If the germination rate in the control sample is less than 90%, an additional preparation step can be applied for treated biowaste material (see Annex A).*

## 8.3 Calculation and expression of results:

The number of all emerged germinated seedlings during the vegetation period is reported. The result is referred to one litre of the original material (equation (1))

$$GP_V = \frac{GP_{sample}}{V_{sample}} \quad (1)$$

where

$GP_V$  is the number of germinated plants per litre sample

$GP_{sample}$  is the number of germinated plants per tray filled with the test sample

$V_{sample}$  is the volume of the tested material per tray in litres

or to 1 m<sup>2</sup> area (equation (2)).

$$GP_A = GP_V \cdot 20 \quad (2)$$

where

$GP_A$  is the number of germinated plants per square meter

$GP_V$  is the number of germinated plants per litre sample

For each replicate, the number of germinated plants is reported. For each tested sample, the mean and the variability of the measurement are calculated. The final result is rounded to one decimal.

## 9 Precision

No data available yet

## 10 Report

The test report shall include the following information:

A reference to the present standard

A complete identification of the sample

Additional treatments (if applied):

Dilution: dilution ratio, EC (EN 13038) before and after diluting

Liming: amount of applied CaCO<sub>3</sub> (g · L<sup>-1</sup>), pH (EN 13037 or ISO 10390) before and after liming

Performing of a washing procedure in the case of a germination rate of less than 90% in the germination control sample (only for treated biowaste)

The laboratory compacted bulk density (EN 13040)

The total number emerged plants per litre of sample or per square metre for each replicate and treatment.

All details not specified in this standard

All incidents which could have had an impact on the result.



## Annex A (normative)

### A.1 Washing procedure for treated biowaste

If the germination rate of the control sample (see 8.2) is less than 90%, this might be due to the presence of substances inhibiting the germination. To reduce these substances, a washing procedure can be applied. The sieved test material (7.1.1) is placed between 2 sieves (500 $\mu\text{m}$ ) in a device permitting a continuous water flow (fig. A1). Water is passed through the test material from bottom to the top at a rate of 10 L · h<sup>-1</sup> for 60 minutes. Afterwards, the material is dried over night at room temperature. The “washed” biowaste material is then submitted to the test as described above again.

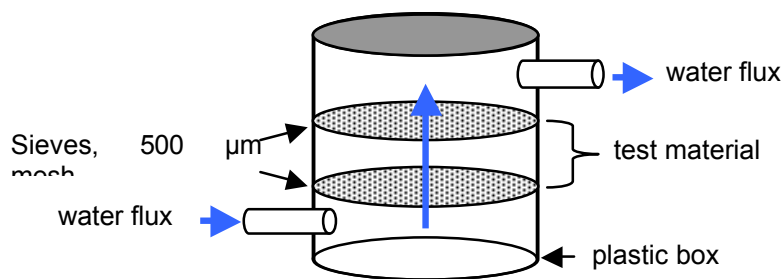


Figure A1: Washing apparatus for performing the washing procedure

**Annex B**  
**(informative)**  
**Validation**

no data available yet

## **Annex C (informative)**

This standard has been developed on the basis of the draft described in the desk study of project HORIZONTAL, followed by a workshop and a further research program. The results and conclusions of this research program are included in the final report "Research program on the methodology for the assessment of the contamination of soil, treated biowaste and sludge with viable plant seeds and propagules"

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