A method to assess viable weed seeds and plant propagules in soils, sludges and treated biowastes.

Eine Methode zur Bestimmung von unerwünschten keimfähigen Samen und austriebsfähigen Pflanzenteilen in Böden, Schlämmen und behandeltem Bioabfall

fr:…..

ICS:

Descriptors:

Document type: European Standard
Document subtype:
Document stage: Working Document
Document language: E

STD Version 2.1c

Contents

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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	☐	[reference]
Sludge	☐	
Soil	☐	

Introduction

Safety warning

1 Scope

This Standard specifies a test procedure for the assessment of contamination by viable plant seeds and propagules on soil, treated biowaste and sludge. Some seeds may require additional treatments to break seed dormancy (see Annex A)

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

ISO 10390 Soil Quality – Determination of pH

ISO 11265 Soil Quality – Determination of the specific electrical conductivity

3 Terms and definitions

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

3.1
test sample
Material after sample preparation as described in clause 7

3.2
Soil (definition to be endorsed)

3.3
Sludge (definition to be endorsed)

3.4
Treated biowaste (definition to be endorsed)

3.5
Weed
any unwanted plant that germinates or emerges

3.6
Plant propagules
parts of plants capable of tillering

4 Principle

The development of plants, whether from seed or plant propagules, is determined after a 21 day 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 as determined by this method

5.3 Calcium carbonate, $CaCO_3$, powdered

5.4 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 Sieve with 20 mm apertures

6.2 Sieve with 5 mm apertures

6.3 Seed tray, height between 50 mm and 100 mm, with bottom perforation

Note: Plastic trays, 430 mm x 330 mm x 60 mm have been found suitable

6.4 Capillary mat

6.5 Perforated plastic sheet

6.6 Thin fleece for covering the trays to avoid air born seed contamination and to assist in the retention of moisture

6.7 Testing facility with temperature monitoring (monitoring range between 18°C and 30°C) and a lighting intensity of at least 10 $W \cdot m^{-2}$ or 2000 lux for 12 hours, e.g. greenhouse, plant growth room

7 Preparation of the sample

[Treated Biowaste

7.1.1 General preparation

Thoroughly mix the sample gently breaking any lump or agglomerate that has been caused by compression, for example, during transportation. Care shall be taken to avoid moisture losses. If necessary, sub-divide the sample to form sub-samples. Sub-sampling may be carried out by any recognised procedure and shall be described in the report.

Pass the sample through a 20 mm sieve (6.1). Any particle which is an intrinsic part of the sample that is retained on the sieve shall be physically reduced in parts of similar size as few times as are necessary to permit the entire sample to pass through the sieve. Thoroughly mix the whole sub-sample with the broken particles that had been retained on the sieve taking care to minimise physical damage to the sample as a whole. Any foreign material such as plastic, metal or glass shall be removed and its content noted.

The volume of the fraction obtained after sieving must be at least 6 litres.

7.1.2 Dilution

One part of the sample material is thoroughly mixed with 2 parts of peat (volume/volume) to produce the test and the control samples. 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 (5.3). 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 EN 13038 is > 50 mS · m^{-1}, the sample has to be further diluted using peat (5.2) until the electrical conductivity does not exceed 50 mS · m^{-1}. For assessing the proper dilution ratio, the electrical conductivity of peat can be assumed to be between 1 and 4 mS · m^{-1}. Diluted samples have to be thoroughly mixed and distributed to an appropriate number of seed trays.

7.2 Soil

Pass the sample through a 5 mm sieve (6.2). with only stones remaining in the retained fraction. Any foreign material such as plastic, metal or glass shall be removed and its content noted. The volume of the fraction obtained after sieving must be at least 6 litres to produce the test and the control samples. 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 (5.3). 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 ISO 11265 is > 50 mS · m^{-1}, the sample has to be diluted using peat (5.2) until the electrical conductivity does not exceed 50 mS · m^{-1}. For assessing of the dilution ratio, the electrical conductivity of peat can be assumed to be between 1 and 4 mS · m^{-1}. Diluted samples have to be thoroughly mixed and distributed to an appropriate number of seed trays.

7.3 Sludge

One part of the sample (at least 3 l) is mixed with 5 parts of peat (at least 15 l) to produce the test and the control sample. Any foreign material such as plastic, metal or glass shall be removed and its content noted. 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 (5.3). If the value is above this range, the material has to be further diluted with peat (5.2). If the electrical conductivity according to EN 13038 is > 50 mS · m^{-1}, the sample has to be diluted using peat (5.2) until the electrical conductivity does not exceed 50 mS · m^{-1}. For assessing of the dilution ratio, the electrical conductivity of peat can be assumed to be between 1 and 4 mS · m^{-1}. Diluted samples have to be thoroughly mixed and distributed to an appropriate number of seed trays.

Note: For solid or coated sludges proceed as described in 7.1

8 Procedure

8.1 Experimental design

The bottom of the perforated seed tray (6.3) is covered by capillary mat (6.4) and a perforated plastic sheet (6.5). Diluted samples have to be thoroughly mixed and distributed to an appropriate number of seed trays. For the test, a minimum of 3 litres of original sample has to be used.

Test sample material is filled into the tray and gently compressed to reach a layer thickness of approx. 20 mm, taking care to ensure uniform compression and depth throughout. A pressing board, cut to the size of the test trays and fitted with a small handle is often useful.

Saturate the diluted test material with water either by watering or sub-irrigation. If using sub-irrigation, the tray has to be immersed for 4 hours in the dark. Afterwards the tray is kept with free drainage until excess water has drained off.

Note: For some seeds, further treatments to break seed dormancy may be necessary. Additionally, a three day storage of the sample at 4°C and/or treatments with Gibberellic Acid or KNO_3 are possible but this is not obligatory (see Annex A).

The trays are kept in the testing facility (6.7) at a temperature suitable for plant germination (range between 18°C and 30°C) without exposure to direct sunlight for 21 days. During the whole testing period, the sample has to be kept moist by watering or sub-irrigation in intervals dependent on plant growth and environmental conditions in accordance with good horticulture practice. In order to reduce desiccation and to avoid air-born seed contamination, the trays are covered with a thin fleece (6.6) as shown in Fig. 1. The germinated plants have to be counted and removed (except if identification is required) once a week.

In Fig. 2, a flow chart of the testing procedure is shown

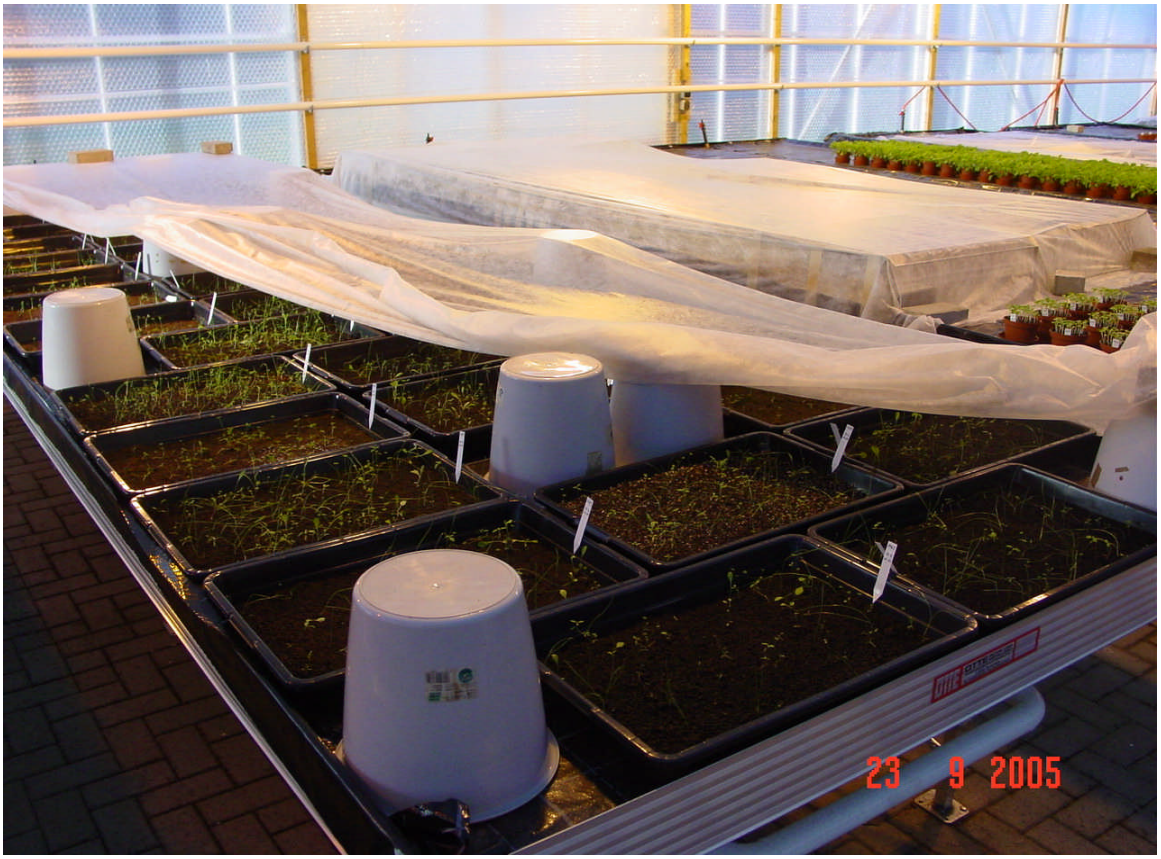

Fig. 1: Cover of the seed trays using a thin fleece

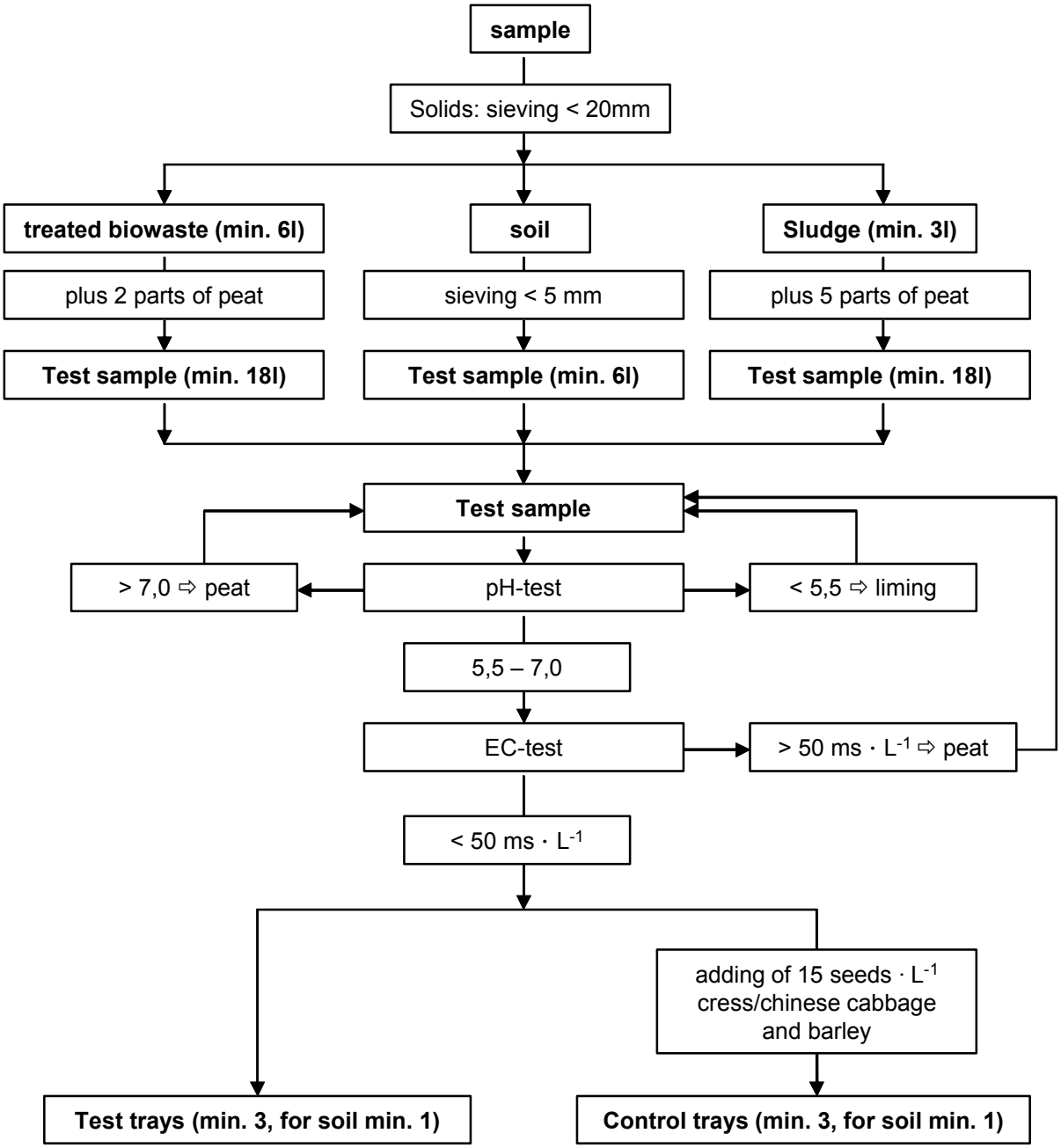

Fig. 2: Flow chart of the testing procedure

8.2 Control sample

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.

8.3 Validity of the test

If the germination rate of cress/Chinese cabbage and barley of the control samples is less than 80%, the results of the test are not valid.

Note If the germination rate of the control sample is less than 80%, a "washing" procedure can be applied for treated biowaste material (see Annex A).

8.4 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 original sample

GP_{sample} is the sum of the total number of germinated plants per original sample

V_{sample} is the volume of the original sample in litres

and can additionally be referred to 1 m^2 area at a defined height (equation (2)).

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

where

GP_A is the number of germinated plants per square meter

GP_V is the number of germinated plants per litre sample

h is the estimated height of the sample in the tray in mm

The final result is rounded to one decimal place.

9 Precision

No data available yet

10 Report

The test report shall include the following information:

A reference to the present standard and, unless use, the sentence: “The results have been obtained without any dormancy breaking treatments”

A complete identification of the sample

Additional treatments (if applied, see Annex A)

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

Liming: amount of applied $CaCO_3$ ($g \cdot L^{-1}$), 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 80% in the germination control sample (only for treated biowaste)

The total number emerged plants per litre of sample and, if additionally requested, per square metre at a defined height, rounded to one decimal.

All details not specified in this standard

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

Annex A (informative)

A.1 Pre-treatment to break seed dormancy

A.1.1 Stratification

The fresh sample is kept at approximately. 4 °C for 72 hours. Afterwards proceed as is described in clause 7 ff. If pre-treatment is applied, it has to be mentioned in the test report.

A.1.2 Treatment with Gibberellic Acid (GA)

For treatment with GA, proceed as is described in clause 8. For the saturation step, use a solution of 1g · L^{-1} gibberellic acid instead of water. If pre-treatment is applied, it has to be mentioned in the test report.

A.1.3 Treatment with KNO_3

For treatment with KNO_3 , proceed as is described in clause 8. For the saturation step, use a solution of 0.01M · L^{-1} KNO_3 instead of water. If pre-treatment is applied, it has to be mentioned in the test report.

Note: A combination of pre-treatment methods is possible.

A.2 Washing procedure for treated biowaste

If the germination rate of the control sample (see 8.2) is less than 80%, 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 (500µm mesh) 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^{-1} for 60 minutes. The out flowing water is passed through a sieve with 50µm mesh to detect seeds with a diameter less than 500 µm (e.g. Juncus sp.). These seeds have to be considered in the test result. Afterwards, the material is dried overnight at room temperature. The “washed” biowaste material is then submitted to the test as described above again.

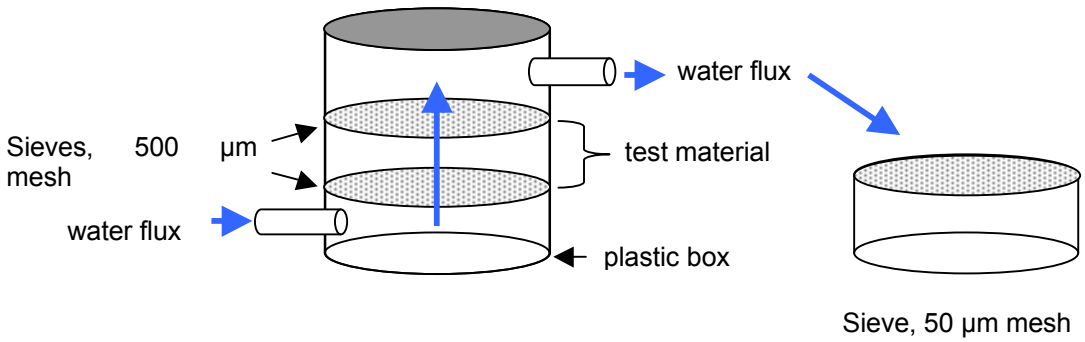

Figure A1: Washing apparatus for performing the washing procedure

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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