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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 —

Méthode pour la détermination des graines germinatives et plantes capables de pousser indésirables dans des sols, boues et déchets biologiques traités —

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Foreword

This document BT/TF151 WI CSS99048 has been prepared by Technical Committee CEN/TC BT 151 “Horizontal”, the secretariat of which is held by DS.

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

This standard is developed in the European project 'HORIZONTAL'. It is the result of a desk study "Horizontal European standard for Contamination with Viable Weed Seeds and Plant Propagules" which aimed at evaluating the latest developments in assessing weeds and Plant Propagules in sludge, soil, treated biowaste and neighbouring fields. After an evaluation study, in which e.g. the ruggedness of the method was studies, a European wide validation of the draft standard has taken place. The results of the desk studies as well as the evaluation and validation studies have been subject to discussions with all parties concerned in CEN. The standard is part of a modular horizontal approach in which the standard belongs to the analytical step.

Until now test methods determining properties of materials were often prepared in Technical Committees (TCs) working on specific products or specific sectors. In those test methods often steps as sampling, extraction, release or other processing, analyses, etc were included. In this approach it was necessary to develop, edit and validate similar procedural steps over and over again for every material or product. Consequently this has resulted in duplication of work. To avoid such duplication of work for parts of a testing procedure references to parts of test methods from other TCs were introduced. However the following problems are often encountered while using references in this way: 1) The referenced parts are often not edited in a way that they could easily be referred to, 2) the referenced parts are often not validated for the other type of material and 3) the updates of such test standards on products might lead to inadequate references.

In the growing amount of product and sector oriented test methods it was recognised that many steps in test procedures are or could be used in test procedures for many products, materials and sectors. It was supposed that, by careful determination of these steps and selection of specific questions within these steps, elements of the test procedure could be described in a way that can be used for all materials and products or for all materials and products with certain specifications.

Based on this hypothesis a horizontal modular approach is being investigated and developed in the project 'Horizontal'. 'Horizontal' means that the methods can be used for a wide range of materials and products with certain properties. 'Modular' means that a test standard developed in this approach concerns a specific step in assessing a property and not the whole ” chain of measurement” (from sampling to analyses). A beneficial feature of this approach is that “modules” can be replaced by better ones without jeopardizing the standard “chain”.

The use of modular horizontal standards implies the drawing of test schemes as well. Before executing a test on a certain material or product to determine certain characteristics it is necessary to draw up a protocol in which the adequate modules are selected and together form the basis for the test procedure.

The modules that relates to this standard are specified in section XX Normative references.

An overview of modules and the manner, in which modules are selected will be worked out later, at which time proper reference in this standard will be provided.

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*

EN ISO 3696, *Water for analytical laboratory use – Specifications and test methods*

ISO 10390, *Soil Quality – Determination of pH*

ISO 11265:1994, *Soil Quality – Determination of the specific electrical conductivity*

ISO 11265/Cor1:1996, *Soil Quality – Determination of the specific electrical conductivity – Technical Corrigendum 1*

CSS99031 Sludge, treated biowaste, and soils in the landscape – Sampling – Framework for the preparation and application of a sampling plan

CSS99058 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 1: Guidance on selection and application of criteria for sampling under various conditions

CSS99057 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 2: Guidance on sampling techniques

CSS99032 Sludge, treated biowaste, and soils in the landscape – Sampling - Part 3: Guidance on sub-sampling in the field

CSS99059 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 4: Guidance on procedures

for sample packaging, storage, preservation, transport and delivery

CSS99060 Sludge, treated biowaste, and soils in the landscape – Sampling – Part 5: Guidance on the process of defining the sampling plan

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 mesh

6.2 Sieve with 5 mm apertures mesh

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

7.1 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 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 $mS \cdot m^{-1}$, the sample has to be further diluted using peat (5.2) until the electrical conductivity does not exceed 50 $mS \cdot m^{-1}$. For assessing the proper dilution ratio, the electrical conductivity of peat can be assumed to be between 1 and 4 $mS \cdot m^{-1}$. 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 mS · m^{-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 mS · m^{-1} and 4 mS · m^{-1}. Diluted samples have to be thoroughly mixed and distributed to an appropriate number of seed trays.

NOTE 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

NOTE 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 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 Figure 1. The germinated plants have to be counted and removed (except if identification is required) once a week.

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

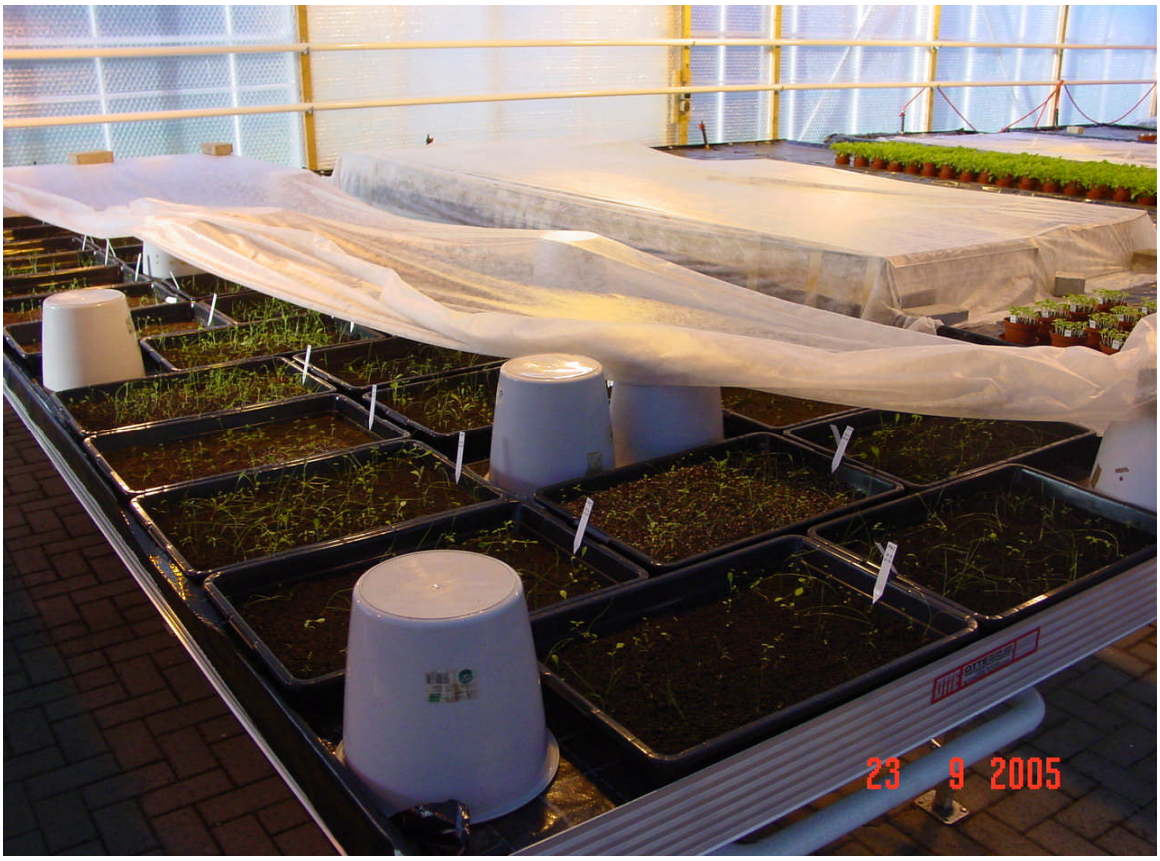

Figure 1 — Cover of the seed trays using a thin fleece

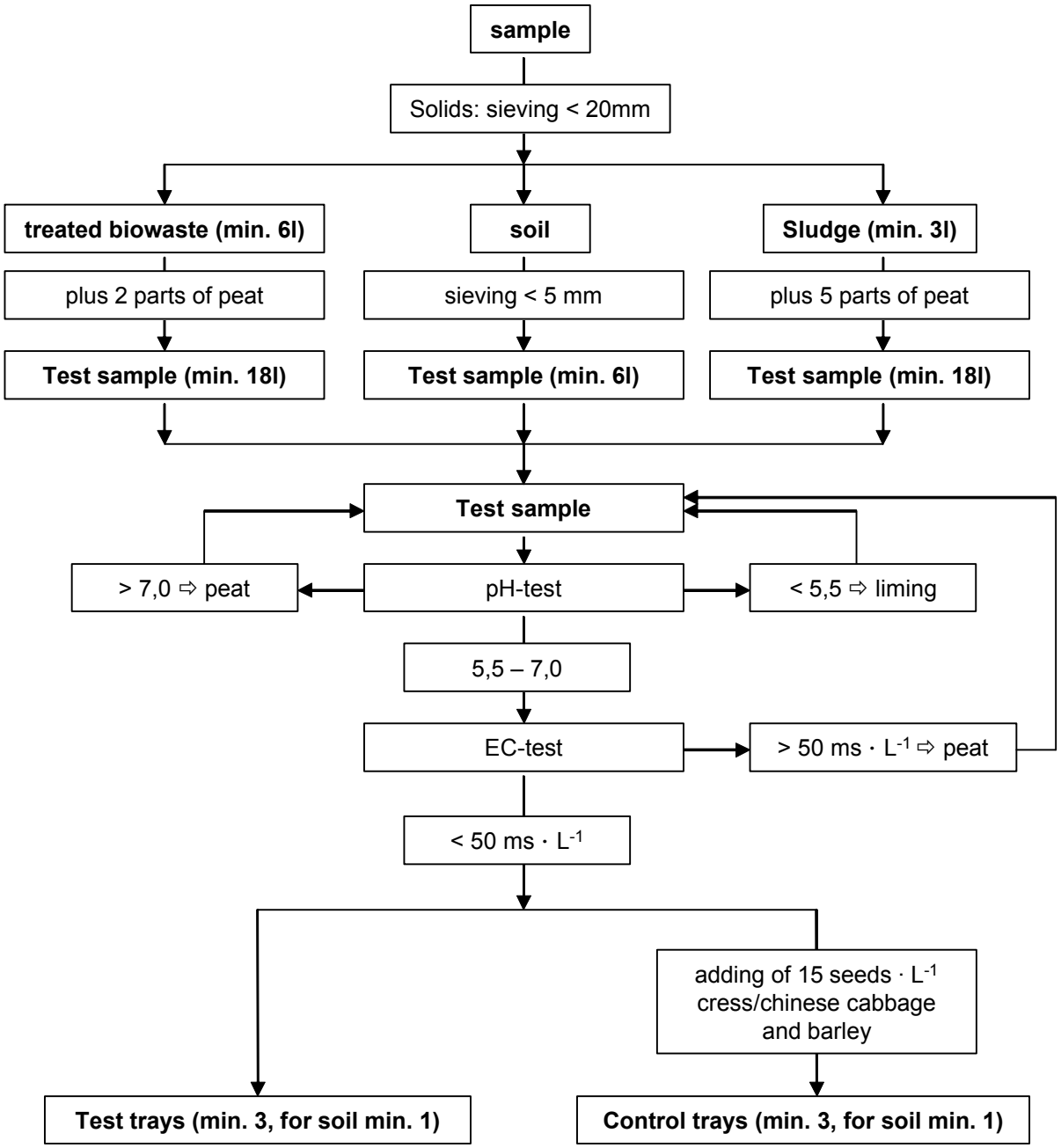

Figure 2 — Flow chart of the testing procedure

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.

NOTE If different trays are used for one sample the seeds should be divided over the trays.

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 % and this was not caused by lack of water or other cultural matters, 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 m2 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 data

The performance characteristics of the method (Annex B) data have been evaluated. Table 2 gives the resulting typical values for repeatability and reproducibility limits as their observed ranges. The typical value is derived from the data in Table B.2 in Annex B by taking the median value and rounding the numbers.

Table 2 — Typical values and observed ranges of the repeatability and reproducibility limits

Results of the validation of a method to assess viable weed seeds and plant propagules in soil, sludge and treated biowaste	Typical value %	Observed range %
Repeatability limit, r %	18	15 - 24
Reproducibility limit, R %	28	23 - 68

NOTE 1. The above results refer to the difference that may be found between two test results performed on two laboratory samples obtained under the same conditions. In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the above typical reproducibility

values and observed reproducibility ranges should be divided by √2 to obtain the corresponding typical dispersion limit and its observed range. In the example of weeds emergence in Compost the result and its dispersion limit is 90.7 ± 14.8 (2 * sR = 16.4 % of 90.7). This means that with a 95 % statistical confidence, the values reasonably attributable to the measured parameter are larger than 76 % and lower than 106 %

NOTE 2. The repeatability limit (r) and the reproducibility limit (R) as given in Table A.2 (Annex A) and in this table are indicative values of the attainable precision if the the method to assess viable weed seeds and plant propagules is performed in accordance with this standard [CSS99048].

NOTE 3. A limited number of materials and parameters were tested. Consequently, for other materials and parameters, performance characteristics may fall outside the limits as derived from the validation of the the method to assess viable weed seeds and plant propagules in soil, sludge and treated biowaste.

NOTE 4 In particular for relatively heterogeneous materials, the repeatability and the reproducibility limits may be larger than the values given in Table B.2 (Annex B) and this table.

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 · 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 (20 mm), 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 1 g · 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 KNO3

For treatment with KNO_3, proceed as is described in clause 8. For the saturation step, use a solution of 0.01 M · 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 (500 µm mesh) in a device permitting a continuous water flow (figure 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 50 µm 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 including the fraction on the sieves is then submitted to the test as described above again.

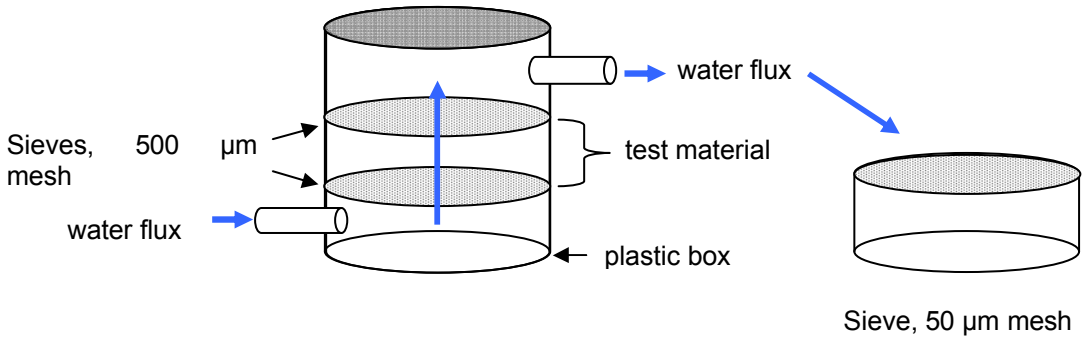

Figure A.1 — Washing apparatus for performing the washing procedure

Annex B
(informative)

Repeatability and reproducibility data

B.1 Performance characteristics

B.1.1 Objective of the interlaboratory comparison

In a European wide interlaboratory comparison study according to ISO 5725-2, the performance characteristics of the standard “method to assess viable weed seeds and plant propagules in soil, sludge and treated biowaste” were established.

B.1.2 Materials used in the interlaboratory comparison study

The interlaboratory comparison of method to assess viable weed seeds and plant propagules in soil, sludge and treated biowaste was carried out with 9 European laboratories on 3 materials. The materials selected for the interlaboratory comparison were chosen to represent soil, sludge and biowaste as broad as possible, because the standard will find general application across different types of soil and soil related materials. (detailed information can be found in the final report on the Interlaboratory comparison study mentioned in the Bibliography).

In the interlaboratory comparison study the following starting points were used:

- The laboratory samples were all taken from one large batch of the different materials according to the normal practice. The normal size reduction and the normal repeated mixing were carried out as needed to obtain representative laboratory samples from the large batch sample (ref JRC).

  Note : the samples provided for the validation should not be confused with reference samples provided for certification purposes, as the performance results obtained have to be directly applicable to daily practice (less rigorous sample preparation than for a reference material).

- The experimental plan was designed by project HORIZONTAL, task group “organic parameters” on the basis of each laboratory being given both a laboratory sample of each material to be tested and seeds with 100% germination capacity. However, in order to verify the contribution to the overall variability of the germination test, the participating laboratories were asked to report the number of trays (repetitions) used and the respective germination counts.

The materials examined cover all the grain size classes to which the the method to assess viable weed seeds and plant propagules in soil, sludge and treated biowaste applies: very fine grained materials (like sludge: 0 µm to about 125 µm) and fine-grained materials (soil and compost: 0 mm to 4 mm).

Table B.1 provides a list of the types of materials chosen for testing and the selected components.

Table B.B.1 — Material types tested and components analysed in the interlaboratory comparison of the method to assess viable weed seeds and plant propagules in soil, sludge and treated biowaste.

	Material type tested	Components analysed[1)]
Solid < 4mm	Biowaste compost	Germination of added seeds pH*, EC*
Solid < 4mm	Soil	Germination of added seeds pH*, EC*
Liquid	Sludge from a digestion plant	Germination of added seeds pH*, EC*

B.1.3 Interlaboratory comparison results

The statistical evaluation was conducted according to ISO 5725-2. The average values, the repeatability standard deviation (s_r) and the reproducibility standard deviation (s_R) were obtained (Table B.2).

The repeatability is determined as an interval around a measurement result (i.e. "repeatability limit"). This interval corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another, both test results being obtained under the following conditions: The tests are performed in accordance with all the requirements of the present standard by the same laboratory using its own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

The repeatability limit was calculated using the relationship : $r_{test} = f \cdot \sqrt{2} \cdot s_{r,test}$ with the critical range factor f = 2.

For instance, the repeatability limit around a measurement result of 90 % emergence of Weeds is ± 16 % (i.e ± 18 % of 90).

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f \cdot \sqrt{2}$ corresponds

to the theoretical factor of 1,96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression of Uncertainty in Measurement (GUM). However it may be necessary to use a larger value for f in situation as described in clause 9.

The reproducibility, like repeatability is also determined as an interval around a measurement result (i.e. "reproducibility limit"). This interval corresponds to the maximum difference that can be expected (with a 95% statistical confidence) between one test result and another test result obtained by another laboratory, both test results being obtained under the following conditions : The tests are performed in accordance with all the requirements of the present standard by two different laboratories using their own facilities and testing laboratory samples obtained from the same primary field sample and prepared under identical procedures.

The reproducibility limit was calculated using the relationship: $R = f \cdot \sqrt{2} \cdot s_R$ with the critical range factor f = 2.

For instance, the reproducibility limit around a measurement result 90 % emergence of weeds ± 25 % (i.e ± 28 % of 90).

NOTE The above relationship refers to the difference that may be found between two measurement results performed each on two laboratory samples obtained under the same conditions. The value f = 2 used in the factor $f \cdot \sqrt{2}$ corresponds to the theoretical factor of 1,96 for a pure normal distribution at 95 % statistical confidence. Also, this value f = 2 corresponds to the usual value k = 2 of the coverage factor recommended in the Guide to the expression of Uncertainty in Measurement (GUM). In the case when reference is made to the dispersion of the values that could reasonably be attributed to the parameter being measured, the dispersion limit is equal to $k \cdot s_R$ with the usual value k = 2, resulting in a dispersion limit lower than the reproducibility limit (i.e. a ratio of $\sqrt{2}$). However it may be necessary to use a larger value $f \cdot \sqrt{2}$ (or k) in situation as described in clause 9 .

In case of relatively heterogeneous materials, the repeatability and the reproducibility limits may be larger than the values given in Tables B.2 (this means that the value chosen for the critical range factor f is larger than 2 as well as for the coverage factor k for dispersion). This is because the extreme results may have been obtained in accordance with the present standard and/or be caused by the variability within, or in between, the laboratory samples.

NOTE For soil, laboratory 5 and 9 obviously included indigenous weeds in the final count, thus resulting in percentages above 100. As the amount of indigenous weeds it is not known, the results have to be regarded as outliers in the first place. Concerning the ability of the lab to provide optimum growing conditions these labs comply as well.

Table B.B.2 — Results of the interlaboratory comparison studies of the method to assess viable weed seeds and plant propagules in soil, sludge and treated biowaste.

Matrix	Parameter	Mean	sr	sR	r	R	p	Outliers	Used number of data
Soil	Weeds	93.1	8.50%	9.23%	22.16	24.08	7	0	19
Sludge	Weeds	77.2	5.19%	24.2%	11.22	52.22	6	0	23
Compost	Weeds	90.7	6.13%	8.17%	15.57	20.75	8	1	31

Abbreviations: sr Repeatability standard deviation;SR Reproducibility standard deviation; r Repeatability limit (comparing two measurements); R Reproducibility limit (comparing two measurements); p Number of labs in statistical analysis.

Note 1. For soil two labs obviously included indigenous weeds in the final count, thus resulting in percentages above 100. As the amount of indigenous weeds it is not known, the results have to be regarded as outliers in the first instance. Concerning the ability of the lab to provide optimum growing conditions these labs comply as well.

Note 2. As different labs worked with different numbers of trays (i.e. repetitions) – this is acceptable due to the method – the calculation of repeatability and reproducibility is not based on the same amount of repetitions in each lab. As the target was to assess the germination rate, first the theoretical amount of plants per tray was calculated, and, based on this, the percentage of plants emerged as compared to the whole amount was expressed. These figures then were used to determine s_r ,r, s_R and R. The values were calculated with respect to the ideal value of 100%. Finally, the amount of labs performing the test in a way that the results can be estimated as valid, seems to be a very important figure as well, but is not foreseen in the template for reporting (compost and soil: 8 our of 9; sludge: 5 out of 8). In the case of sludge, a main problem still seems to be the preparation of the test sample – a task that can be covered by a respective training course together with methods for sample pre-treatment.

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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