

Contamination with Viable Weed Seeds and Plant Propagules

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CONTENTS

LIST OF TABLES	4
SUMMARY	5
1. INTRODUCTION	6
2. EXISTING METHODOLOGY	7
3. EVALUATION OF DRAFTING A HORIZONTAL STANDARD	8
3.1 Dilution	8
3.2 pH- and Nutrient Status of the tested Material	9
3.3 Adjustment of Moisture Content before and during the experiment	9
3.4 Sample size and performance of the growing test	9
3.5 Stimulation of germination by low temperature stimulus	10
3.6 Growing Conditions	10
4. CRITICAL POINT AND RECOMMENDATIONS	11
4.1 General Remark	11
4.3. Method recommendations	11
4.4. Performance of watering	11
5. DRAFT STANDARD (CEN TEMPLATE)	12
5.1 Scope	12
5.2 Normative references	12
5.3 Terms and Definitions	12
5.4 Principle	12
5.5 Reagents	12
5.5.1 Inert Diluent: <i>quartz sand or washed perlite or</i>	12
5.5.2 Calcium carbonate	12
5.6 Apparatus	13
5.6.1 Seed tray (e.g. Styrofoam box), app. 540 mm x 400 mm x 80 mm	13
5.6.2 Glass plate or plastic for covering the tray	13
5.6.3 Temperature room or equivalent (e.g. refrigerator) at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$	13
5.6.4 Testing facility: <i>plant growth room (phytotron) or greenhouse or only shaded outdoor conditions during normal vegetation period</i>	13
5.7 Preparation of the sample	13
5.8 Procedure	13
5.8.1 Experimental design	13
5.8.2 Calculation and Expression of results:	13
5.9 Precision	14
5.10 Test report	14
REFERENCES	15

LIST OF TABLES

Table 1: Sample size and some other performance characteristics	9
Table 2: Growing conditions of the mentioned methods and procedures	10

SUMMARY

EU directives require a reduction in landfill and where possible recycling of waste in the form of composted material. Especially if these materials are used for remediation or as a constituent for growing media or for improving soil fertility with considerable nutrient effects on arable land, contamination with germinating weed seeds is an important quality parameter.

There are only draft standard methods, but several methods are in use differing mostly due to the substrate they are dealing with. Furthermore different treatments (dilution, pH-adjustment, fertilizing, low temperature stimulus, ...) of the tested material and afterwards of the growing conditions, which all might affect growing rate, were stressed.

In the proposed draft standard, the most suitable conditions are tried to be combined.

1. INTRODUCTION

The contamination of horticultural substrates, soil improvers, growing media, sludge or composts with viable weed seeds and sprouting plant parts is of special importance if these materials are used for the following purposes:

- Remediation of landscape
- Constituents of growing media
- Application on arable land for improving soil fertility and considerable nutrient release from the organic material for fertilizing

The test for germinating weed seeds has mainly been developed for materials used as constituents for growing media. Most of the methods described deal with compost, composted material and peat. Especially when the composting management was not optimal and the rise in temperature was not done uniformly, viable weed seeds still are there in composts and emerge after application. The proposed draft tries to combine all the important factors mentioned in the different approaches to allow a widespread use.

2. EXISTING METHODOLOGY

There is no existing standard for the assessment of weeds and plant propagules. The mentioned Austrian standard is a draft.

VDLUFA: Methods Book I: Proof of germinating weed seeds and plant propagules in horticultural substrates and original materials for substrates (in agreement with the Dutch RHP).

ÖNORM S 2021 (Draft): Growing media – Quality requirements and test methods

IPS (International Peat Society), **HOPE**: Draft method for the determination of weeds in raised bog peat (Bord Na Mona, Ireland) -

WRAP (The Waste and Resources Action Programme): The Composting Association (2002): Standards for Composts Test method. Public Available Specification 100 – Specification for composted material, Annex D: A Method to assess contamination by weed propagules and phytotoxins in composted material (UK)

FCQAA: Federal Compost Quality Assurance Association (Germany), Methods Book for the Analysis of Compost, 4. Edition 1998: Determination and evaluation of the content of germinating seeds and plant propagules (state approved RAL-commercial norm of the producers of compost and is prescribed by executive order law: Biowaste Ordinance)

The methods mentioned below have been supplied by organisations other than standardisation bodies, especially dealing with composts :

Rijkslaboratorium Gent (Belgium): Method Germinative Seeds or Weed Contamination

BRL-K10001, 2002. Substrate materials, Set of appendices on analytical methods for the product certificate for Substrate materials. Kiwa N.V., Rijswijk The Netherlands.

L. **Faessel** (Rittmo): New method proposed for the detection of weeds seeds in composts.(unfortunately we are not able to find the correct quotation)

VITO, (1996): Standard Operating Procedure: Method Germinative Seeds or Weed Contamination (Kiemkrachtige Zaden).

Furthermore, there are national laws dealing with weed seeds contamination:

ACO: Austrian Compost Ordinance (Effective Date: Sept 1, 2001): Annex 5: Test for germinating seeds and plant propagules.

GOUBW: German Ordinance on the Utilization of Bio-Wastes on land used for agricultural, silvicultural and horticultural purposes (Biowaste Ordinance - Bioabfallverordnung), 1998

3. EVALUATION OF DRAFTING A HORIZONTAL STANDARD

The principle of all mentioned drafts, methods and procedures is the same: Detection of viable weed seeds or plant propagules by stimulating the germination of the seeds and the sprouting of plant propagules in the material of interest under standardized preparing and optimal growing conditions over a period of two to four weeks.

First of all, as mentioned before, the different materials of interest under investigation should be referred:

- Bio-Wastes such as compost (or sewage sludge): WRAP, FCQAA, Rijkslabo Gent, Faessel, VITO, ACO, GOUBW
- Original materials, e.g. peat, as constituents for growing media or soil improver: VDLUFA, IPS
- Growing media and horticultural substrates: VDLUFA, ÖNORM S 2021

The most interesting material concerning contamination with viable seeds and sprouting plant propagules is compost.

Although the principle of detection the seeds and plant propagules is the same, several factors concerning the standardization of preparation and the optimal growing conditions should be considered:

3.1 Dilution

Dilution of the tested material is necessary, if chemical properties, especially the salt content expressed as electric conductivity, might inhibit the germination and the sprouting. In this case, the substrate has to be diluted using suitable diluents.

- ÖNORM S2021: When salt content is > 3 g per litre in the testing material a dilution using quartz sand (recommended in the ratio of 1:1) is provided.
- WRAP: In the preparation of the test sample a dilution with an equal volume of sphagnum peat is provided.
- FCQAA: When salt content is > 2 g per litre in the testing material a dilution using peat added with 4 g calcium carbonate per litre is provided.
- Rijkslabo Gent: 500 ml of the fresh sample should be mixed with 2000 ml white peat.
- Faessel: After washing of 0,4 l of fresh material with deionized water a mixture with 0,8 l of non fertilised blond peat is prepared (by washing a reduction or elimination of soluble anti-germinative compounds which are released by microbial activity during composting might occur)
- ACO: Electric conductivity should be $< 1,7$ mS/cm by dilution using quartz sand.

To overcome this problem it seems to be most appropriate to use the determination of the salt content as electric conductivity as described in CEN 13037. However, up to now there is only little experience in the relationship between the values described above and the corresponding results according to CEN 13037. Therefore, research work in this field will be necessary.

3.2 pH- and Nutrient Status of the tested Material

- VDLUFA: When peat is tested the determination of pH and the adjustment of a pH > 5,5 by liming and also the supply of 1 g per litre of a multi nutrient fertilizer is provided.
- IPS: 1 g fertilizer per litre is added to the peat. In case of weakly decomposed peat 5 g/l of calcium carbonate are added, for strongly decomposed peat 9 g/l. The pH of the peat medium shall be between 5,0 and 6,0.
- WRAP: Determination of water soluble nitrate and ammonium of the sample. If necessary, addition of calcium nitrate is provided to bring the total water soluble nitrogen content to 240 mg/l.

3.3 Adjustment of Moisture Content before and during the experiment

Due to the diversity of physical characteristics of the different materials it is hardly possible to define a specific moisture content for conducting the experiment, especially because different methods for the determination of the Water Holding Capacity (WHC) are in use. However, 100% WHC with or without drainage does not seem to be appropriate for all the materials to be tested. As a consequence, the moisture content is often not specified in detail.

- VDLUFA, ÖNORM S 2021, IPS, VITO, ACO: No specific recommendations, optimal moisture content for cultivating should be observed. Covering of the tray by plastic plane or glass plate at the beginning is provided for a week to ten days.
- WRAP: The tested material in the trays is placed in water until thoroughly wet, afterwards excess water is allowed to drain away, the trays are placed on moist capillary matting covered with opaque plastic till the seedlings appear.
- FCQAA: At the beginning of growing experiment the tested material is watered at total holding capacity, afterwards a cover plate of glass or plastic may be used to avoid desiccation. When the covering is removed spraying of water is recommended.
- Faessel: The recipient containing the tested mixture is placed in a larger recipient that contains a solution of gibberelic acid (500 mg/kg) so that the compost will be immersed in gibberelic acid for 15 min.

3.4 Sample size and performance of the growing test

To achieve representative results also replications and control samples should be taken into consideration. Greater sample size and replicates may improve the reliability of the results. Also the layer thickness of the tested material filled into the trays varies widely; thereby the germination rate might be influenced.

Table 1: Sample size and some other performance characteristics

	Sample size	Layer	Replications	Control sample	Result
VDLUFA	3 litre	2 cm	No	No	Plants/litre
ÖNORM S 2021	3 litre	1-2 cm	No	Yes ¹⁾	plants/litre
IPS	3 litre	4-5 cm	No	No	Plants/m ²
WRAP	2 litre	n.s. ²⁾	No	Yes	Plants/litre
FCQAA	3 litre	1 cm	No	No	Plants/litre
Rijkslabo Gent	0,5 litre	2-3 cm	No	Yes ³⁾	Plants/litre
Faessel	0,4 litre	3-4 cm	3	Yes ⁴⁾	Plants
VITO	2,5 litre	2-3 cm	No	Yes	Plants
ACO	3 litre	1-2 cm	No	No	Plants/litre

- 1) For detecting local wind borne weed contamination when necessary a sterile control is provided, in closed growing chambers controls might be omitted.
- 2) Not specified (about 1-2 cm)
- 3) Negative control of white peat due to mixing the tested sample with this material
- 4) Positive control seeds of barley and cress

3.5 Stimulation of germination by low temperature stimulus

The physiological phenomenon of seeds dormancy may be raised by physical treatments e.g. by a low temperate stimulus under wet cool conditions at 4°C for 3 days. In the procedures of VDLUFA; ÖNORM S 2021; Faessel and ACO this treatment is conducted explicitly.

3.6 Growing Conditions

Also the growing conditions during the experimental period may influence germination rate: As it can be seen in Table 2 the mentioned conditions are often rather unspecific, also the duration varies from two to four weeks.

Table 2: Growing conditions of the mentioned methods and procedures

	Facility	Temperature	Relative Humidity	Light specification	Day length	Duration
VDLUFA	Green house, Growth chamber	18 – 25°C	n.s.	3000 lx	16 hours	2–4 weeks
ÖNORM S 2021	n.s.	20°C	n.s.	No direct sun radiation.	n.s.	3 weeks
IPS	Green house, Growth chamber	18 – 25°C	n.s.	Additional lighting in winter	n.s.	4 weeks
WRAP	Green house, Growth chamber	15 – 25°C	n.s.	n.s.	n.s.	4 weeks
FCQAA	n.s.	18 - 20°C	n.s.	> 1000 lx	n.s.	15 days
Rijkslabo Gent	n.s.	21°C	Nearly 100%	n.s.	n.s.	2–3 weeks
Faessel	Phytotron	25°C/20°C	90%/70%	50 W/m ²	15 hours	3 weeks
VITO	n.s.	21°C	Nearly 100%	n.s.	n.s.	2–3 weeks
ACO	n.s.	20°C	n.s.	No direct sun radiation	n.s.	2 weeks

n.s. not specified

4. CRITICAL POINT AND RECOMMENDATIONS

The mentioned methods and procedures differ in the most relevant factors. It seems still uncertain, if there is the necessity for a sophisticated method including previous determination of salt content, pH, nutrient status, water supply and exactly defined growing conditions (temperature, relative humidity, day length and defined light radiation, which only can be provided in a growth chamber) or a quite simple method, which can also be conducted using rather simple equipment normally available by producers of substrates and composts.

4.1 General Remark

First it should be evaluated if the different mixing procedures and other pre-treatments affect significantly the results. In a French study about the reproducibility for the detection of adventitious seeds four combinations of eight different treatments were tested. Especially stratification (the humidified test material is placed at 4°C during 72 h), gibberellic acid treatment, compost washing and dilution with peat improved germination in compost by 58, 48, 46 and 38% compared to control. Also the types of the seeds (e.g. mono- or dicotyledonous) might be influenced in different ways by the pre-treatments. The proposed procedure significantly improved the germination rate in artificially contaminated compost.

Secondly the same must be achieved for the growing conditions. Especially the requirement of a growth chamber where temperature, humidity and day length can be adjusted must be proved clearly. A standard method should give reliable results at standardized operating procedures and growing conditions, which should be as simple as possible.

4.2. Available parameters

As relevant factors influencing germination such as pH or salt content are standard parameters in analysis of composts or substrates and therefore available, threshold values (according to CEN 13037) for salt content/electric conductivity should be determined. At least these parameters should be included in the test reports.

4.3. Method recommendations

When dilution is performed the general use of a suitable standardized diluent which is commonly available is proposed. Appliance of the method for samples with high content of large particles (> 40 mm) and appropriate particle size for conducting the experiment should be evaluated.

4.4. Performance of watering

As has been pointed out earlier, it is hardly possible to define specific moisture content or precise watering conditions during the performance of the experiment. Therefore it is recommended to evaluate the influence of different moisture conditions and the thickness of layer of the tested hat on germination rate.

5. DRAFT STANDARD (CEN TEMPLATE)

NOTE: Where italics appear in the draft method it indicates an area that requires additional work and confirmation

5.1 Scope

This Standard specifies a test procedure for the assessment of contamination by germinating weeds and plant propagules on various substrates.

5.2 Normative references

This method 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 method only when incorporated in it by amendment or revision. For undated references the latest edition of the publications referred to apply.

EN 13037 Soil improvers and growing media – Determination of electric 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

5.3 Terms and Definitions

To be defined.

5.4 Principle

The development of weeds, whether from seed or other plant propagules is determined after a 4 week incubation period under controlled conditions

5.5 Reagents

5.5.1 Inert Diluent: *quartz sand or washed perlite or peat*

5.5.2 Calcium carbonate

5.5.3 Multi Fertilizer

5.6 Apparatus

5.6.1 Seed tray (e.g. Styrofoam box), app. 540 mm x 400 mm x 80 mm

5.6.2 Glass plate or plastic for covering the tray

5.6.3 Temperature room or equivalent (e.g. refrigerator) at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$

5.6.4 Testing facility: *plant growth room (phytotron) or greenhouse or only shaded outdoor conditions during normal vegetation period*

5.7 Preparation of the sample

The sample preparation has to be carried out in accordance with EN 13040, clause 8. For this test, *material* < 20mm is used.

5.8 Procedure

5.8.1 Experimental design

3 l of sample material are filled in the seed tray in a layer of 2 cm (5.6.1). If the electric conductivity according to CEN 13038 is > 50 mS/m, the sample has to be diluted using quartz sand or washed perlite until the electric conductivity does not exceed $50 \text{ mS/m} \pm 2,5 \text{ mS/m}$. For calculation of the dilution ratio, the electric conductivity of the mixing component is assumed to be 0 mS/m. After diluting, the electric conductivity has to be checked again.

Diluted samples have to be thoroughly mixed and distributed to a respective number of seed trays, e.g. if using a dilution ratio of 1 + 1 (v/v) of sample and sand, two trays containing 1,5 l sample and 1,5 l sand each have to be used.

If necessary, the test sample has to be moistened. Consequently, the seed tray is covered by a glass plate and kept at 4°C for three days (wet cold stimulus of germination). Afterwards, the tray is kept in the testing facility (*phytotron, plant growth room or greenhouse or ...*) at a temperature suitable for plant germination without exposure to direct sunlight. After 10 days or earlier at emergence of the first seedlings the glass plate is removed, leaving the tray exposed to day-light for a further 18 days. The sample has to be kept moist during the whole period by daily spraying.. The number of germinated plants has to be recorded three times weekly to avoid the missing of plants caused by, for example damping off. After this second exposition period, the total number of germinated plants is recorded and referred to 1 litre of the original substrate.

To reveal possible local contamination during the experiment (outdoor), a control sample has to be treated in the same way as the test sample. If using closed environments like phytotrons, the control sample is not obligatory. The experiment has to be performed in triplicate.

5.8.2 Calculation and Expression of results:

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

$$GP = \frac{GP_{sample} - GP_{control}}{3} \quad (1)$$

where

GP is the number of germinated plants per litre sample

GP_{sample} is in the trays filled with sample material

$GP_{control}$ is the number of germinated plants in the trays filled with the control material

The final result is rounded to one decimal

5.9 Precision

No data available at the moment

5.10 Test report

The test report shall include the following information:

A reference to this method

A complete identification of the sample

The EC figure according to EN 13037 before and after diluting

The kind of diluent

The dilution ratio

The total number emerged weed propagules per litre of sample

REFERENCES

ISO/CD 17126: Soil quality – Determination of the effects of pollutants on soil flora – Seedling emergence, screening test with lettuce (*Lactuca sativa* (L.))

ÖNORM S 2021 (draft): Requirements for growing media

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VDLUFA: Proof of viable Seeds and sprouting plant propagules in horticultural substrates and in original materials for substrates. Methods Book I, A 13.5.2

Rijkslaboratorium Gent: Method germinative Seed or Weed Contamination