Research program on the methodology for the assessment of the contamination of soil, treated biowaste and sludge with viable plant seeds and propagules

Final Report of the ruggedness test

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Austrian Agency for Health and Food Safety

In co-operation with:
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G. Schmilewski (Klasmann-Deilmann, Germany)
E. Pfundtner (AGES, Austria)

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Draft Method

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Contents

1. Introduction

Following the desk study report for the assessment of weed seeds and viable plant propagules a workshop was held in Vienna (November 2004) to enable interested experts to express their opinions and suggest improvements. At the conclusion of the workshop, the participants agreed, the participants agreed the general principle of the method, but pointed out the following facts that still needed clarification prior to a draft standard method.

1.1. Handling of liquid sludge

It was agreed that the method of test was not applicable to liquid sludge. Thus, this matrix has to be inserted into the scope of the method and a proper description of handling has to be added.

1.2. Material of dilution

Discussion took place as to what material should be used to dilute the test sample. It was generally agreed that dilution was necessary as the most composted wastes and sludge's would not support plant emergence due to high conductivity and a possibility that the pore structure would not be satisfactory. Various diluents were suggested for example soil, peat, perlite and vermiculite but no agreement was reached as to the most appropriate – it may be that more than one diluent is required.
It was agreed that research is required into the effects of various diluents and ways of ensuring that the diluents are weed free. Furthermore, a large proportion of the original sample should be tested.

1.3. EC threshold

No agreement was reached as to the "best" EC threshold. There is some knowledge of threshold values for plants but no one was sure that all weeds would germinate under the same conditions.
It was agreed that it is necessary to undertake a trial with various EC's and weed seeds.

1.4. Additional treatments

It is an established fact that some seeds require either cold or heat to stimulate germination. In view of the wide range of climatic conditions within the EU both aspects must be considered.
It was agreed to test several methods to break a possible dormancy and to propose the most appropriate one as an additional treatment.

1.5. Moisture and watering regime

This may appear a simple subject but over- or under-watering can have a significant impact on the result. As so many different sample types are to be included this area needs investigating – different regimes may be required for different matrices.
It was agreed to investigate possible effects of different watering regimes with respect to the emergence of seeds

1.6. Thickness of the layer

No detailed information was available concerning the influence of the thickness of the layer. Therefore it was agreed to determine the optimum thickness.

1.7. Size of test tray

This question may appear of minor significance but can have a cost implication. However, it has to be seen in context with clause 1.2 and 1.6, which shall be the basis of the final decision for the tray size.

1.8. Duration of the test

As not exact data is available concerning the optimum duration of the test, it was agreed that whilst research was being carried out records should be kept on weed seed emergence. From this data the longest time necessary to undertake the test shall be obtained.

1.9. Environmental Conditions

Furthermore it was agreed to keep the general conditions as lighting requirements, water regime and temperature as simple as possible.

1.10. Research matrix

The following laboratories volunteered to take part in the research work compiled in the study matrix (table 1):

- Institute for Soil Health and Plant Nutrition, Austrian Agency for Health and Food Safety (AGES)
- Recherche Innovation et Transfert de Technologie pour les Matières fertilisantes Organiques (RITTMO)
- Institut für Gartenbau, Fachhochschule Weihenstephan – Versuchsanstalt für Gartenbau
- Klasmann – Deilmann GmbH

Based on the results of the research work a revised draft standard has been prepared that will serve as the basis for the forthcoming validation tests. The main results of the studies undertaken by the above named laboratories are presented and conclusions for the final draft are drawn. Additionally, the studies are attached in full length (the work done in Weihenstephan is only available in German language, the work done at AGES is incorporated in the text).

Table 1: Study matrix

	laboratory			
test parameters	RITTMO	Klasmann-Deilmann	Weihenstephan	AGES
test material		composted biowaste, raised bog peat	PBGM, topsoil, green waste compost	sludge, biowaste-compost, topsoil
dilution material	peat, soil, sand	bog peat (H3-H5), perlite	peat, perlite	peat, sand
EC threshold	measured	measured	measured	measured
pH, nutrient status	measured	measured	measured	measured
Additional treatments	several dormancy breaking procedures	no	no	no
moisture	subirrigation	over head watering	over head watering	spraying
Thickness of the layer		1, 2, 4 cm	1, 2, 3, 4, 5 cm	2, 4 cm
Size of the tray	varying	60 x 40 cm	56 x 36,5 x 6 cm	43 x 33 x 6 cm
weeds	10 different species	10 commonly found weeds	11 commonly found weeds	Lepidium sativum, Lolium multiflorum, Chemopodium album
duration	recorded	recorded	recorded	recorded

2. RITTMO study: Weeds ruggedness test

2.1. Introduction

The study performed in France was focussed on the following problems:

- Selection of dilution materials: peat, soil and sand
- Additional treatments: washing, sieving,
- Use of commercially available seeds (cress, tomato and ryegrass) and seeds sampled in nature.
- Treatments for breaking seed dormancy: gibberellic acid, stratification, sulphuric acid, ethanol, potassium nitrate, soaking, lixiviation.

The matrices used for the investigation were different origins of compost and soil.

2.2. Methods for breaking tegumentary seed dormancy of commercially available seeds – liquid treatment

In the course of this test, seeds were mixed into 45g of substrate, placed in a container and submitted to the following treatments by immersing the substrate for 16h:

- Gibberellic acid (GA): 1g · L^{-1}
- Ethanol: 10% and 5%
- Sulphuric acid: 1% and 0,5% (v/v)
- Water (Control)

The GA-treatment gave results comparable to the control, Ethanol and sulphuric acid resulted in a decrease of seed germination

2.3. Methods for breaking tegumentary seed dormancy of commercially available seeds – Stratification

After a treatment with GA, seeds sown on weakly decomposed raised bog peat were kept at 4°C for 3, 7 and 14 days. The best result of emergence (average and variability) was reached after 3 days stratification.

2.4. Effects of different treatments on natural weed seeds

Green waste compost artificially contaminated with various seeds was treated according to the following modalities:

- Dilution with peat: 1 part compost + 2 parts peat
- "Washing": 1l compost with 4l water
- Stratification: 72h at 4°C
- Gibberellic Acid (GA): 50ppm, 15 minutes
- Soaking in deionised water (4 h)
- Ethanol (4.5%)
- Sulphuric acid (3%)

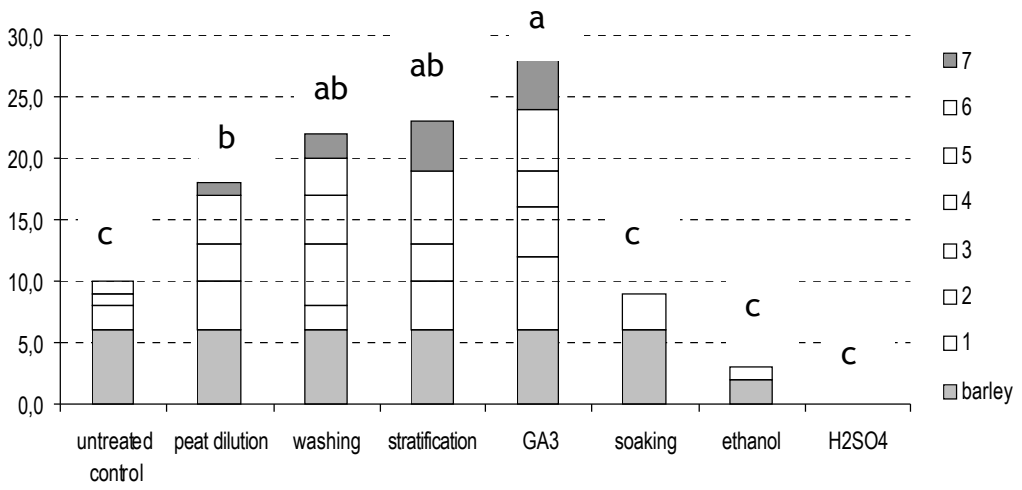

Figure 1: effect of individual treatments on natural weeds seeds germination; differing letters showing significant differences, numbers representing different plant species

Different seeds were differently influenced, but summarizing the effect of the GA-treatment showed the most obvious – and also in most cases significant - improvement.

A combination of treatments, namely

- treatment w : washing / dilution with peat / gibberellic acid / stratification;
- treatment x : washing / gibberellic acid / stratification;
- treatment y : washing / dilution with peat / stratification;
- treatment z : washing / dilution with peat / gibberellic acid

showed the improvement of germination as compared to the control (Fig. 2).

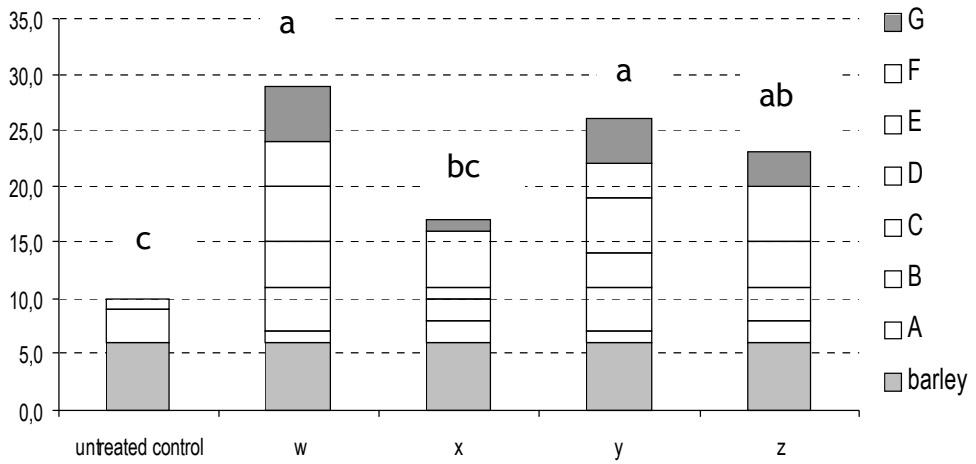

Figure 2: Effect of treatments combination on natural weeds seeds germination; differing letters showing significant differences, Capital letters representing different plant species

Despite the improvement by the different combinations, not all seed types (type D) were able to germinate. However, treatments seem to be necessary to break seed dormancy in composted material.

2.5. Studies with soil naturally containing weed seeds

Three different methods used in Europe for the assessment of weed seeds were compared:

- RITTMO – method: dilution with weakly decomposed raised bog peat, subirrigation with GA (15 minutes) and stratification (72 h), 4 cm layer
- Austrian compost ordinance: material ≤ 10mm; dilution with silica sand, 3 days stratification (4°C), covered for one week by a glass plate
- German method for growing media according to VDLUFA: material ≤ 4mm; adjustment of pH, adding of fertilizer, 2 cm layer

The experiment was conducted using a mixture of mature greenwaste compost and soil that contained many naturally occurring weed seeds.

After 24 days of culture and depending on the replication, for 1 litre of the tested material up to 10 emerged seedlings were observed with the RITTMO method, 0.4 with the German method and none with the Austrian method.

Secondly, an additional treatment with KNO_3 was tested. A combination of 0,01 mol · L^{-1} KNO_3 with GA – treatment showed the best germination rates.

2.6. Reduction of electric conductivity (EC)

Soaking, peat dilution and lixiviation were tested to compare their impact on electrical conductivity of two materials, namely vermicompost (EC: 106 mS · m^{-1}) and sieved pig manure compost (EC: 327 mS · m^{-1})

- Soaking: water was supplied by subirrigation for 4 or 24 hours.
- Weakly decomposed raised bog peat dilution: after soaking, 1 volume-part of compost was mixed with 2 volume-parts of weakly decomposed raised bog peat.
- Lixiviation: composts were placed between 2 sieves (500µm) in a plastic box, water was passed through it upwards.

The described lixivation procedure led to a fast and pronounced reduction of EC. However, peat dilution led to reasonable results as well, although increasing the amount of material to be tested.

2.7. Conclusions

- Results obtained exhibited that the percentage of germination is very much dependent on the type of the seeds. Naturally occurring weed seeds appeared to be much more reactive than commercially available seeds to treatments applied to break seed dormancy. This is probably due to the lacking dormancy of commercial seeds, thus having a satisfying percentage

of germination. However, most of seeds present in soils, soil improvers and growing media correspond to the behaviour of natural weeds seeds.

- The best improvement of germination could be reached by a combination of washing, dilution with peat, gibberellic acid treatment and stratification.
- Addition of 0.01 mol · L^{-1} KNO_3 to gibberellic acid in the combination of treatments described above might further improve the percentage of weeds seeds germination. Replacing the initial washing step of this above combination of treatments by lixiviation might be useful in case of high initial electrical conductivity of tested material.
- Further improvement of the test might be obtained by addition of stratification treatments appropriate to remove tegumentary dormancy. The integration of an additional "positive control", i.e. test material amended with seeds sensitive to "antigerminative" molecules is suggested.

Comment:
Due to the fact that the new standard should be kept rather simple to allow a widespread performance, of the treatments described above only the most effective have been incorporated into the final draft method.

3. AGES – study: Assessment of substrate contamination with viable weed seeds

3.1. Introduction

The Austrian study was focussed on the following problems:

- Description of a proper procedure for the use of sludge
- Dilution material (sand, peat)
- EC threshold
- Layer thickness

3.2. Materials and methods

The solid test sample was a sieved mixed green- and biowaste compost from a rural area in lower Austria sieved. The liquid substrate used in that experiment originated from a biogas plant, which is operated with maize and grass silage. The tested compost with an initial salt content of 240 mS · m^{-1} (appr. 8,5 g · L^{-1} KCl) was diluted with peat (1 part compost with 2 parts peat, v/v) and with quartz sand in two mixing ratios (1 + 2 mass/mass and 1 + 2 volume/volume). The digestate (300 ml) was mixed with a control substrate (peat based growing medium and tennis court sand, 1 + 1 m/m). For the digestate, the control substrate was mechanically mixed with the liquid (10 + 1, v/v) using a clay mixer (see figure 3).

Figure 3: Mixing of control substrate and digestate

Soil (dried and sieved ≤ 4 mm) sampled from the surface layer (0-10 cm) of arable land was also tested. The reduction of EC according to dilution is presented in Table 2.

Table 2: EC (EN 13038) of initial substrates and diluted mixtures

Material	EC mS · m^{-1}
Pure Compost	226
Compost + Peat (1+2 v/v)	88
Compost + Sand (1+2 m/m)	62
Compost + Sand (1+2 v/v)	44
Control substrate	32
Control + digestate (10 + 1 v/v)	45
Soil	76

All test substrates were put homogeneously in layers of 2 cm or 4 cm thickness, corresponding to approximately 3 and 6 litres material, in commercially available plastic trays (dimension: 43 x 33 x 6 cm) with bottom perforation covered with a capillary mat.
Seeds of the following species were tested:

Lepidium sativum L. ssp. sativum (cress),
Lolium multiflorum Lam. (Italian Raygrass) and
Chenopodium album L. (meldweed)

The seeds were mixed into the test substrates before the trays were filled. The test trays were set up in the greenhouse over a period of 38 days with an illumination intensity of at least 2000 lux and a room temperature of 18°C to 24°C without direct sunshine. The water loss was regularly compensated by spraying. The germinated plants per tray were counted three times per week.

3.3. Results

The germination rates ranged from 30% (meldweed) to 100% (cress). Figure 3 and 4 show the response of the plants to the different substrates and layers:

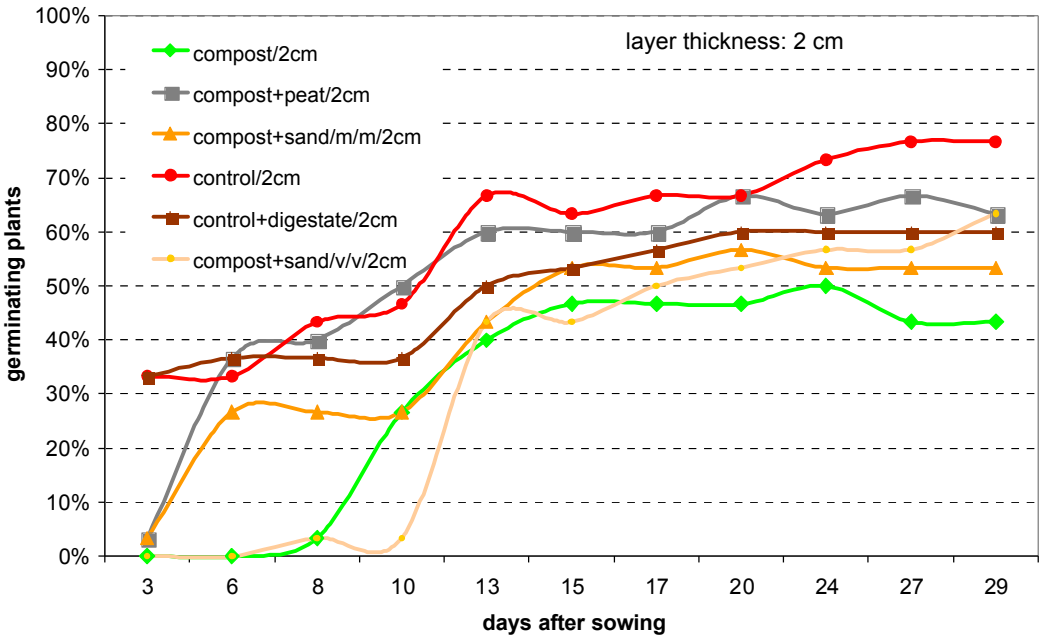

Fig. 4: Germination rate of the three test crops (mean) in compost depending on the dilution material in a 2 cm layer

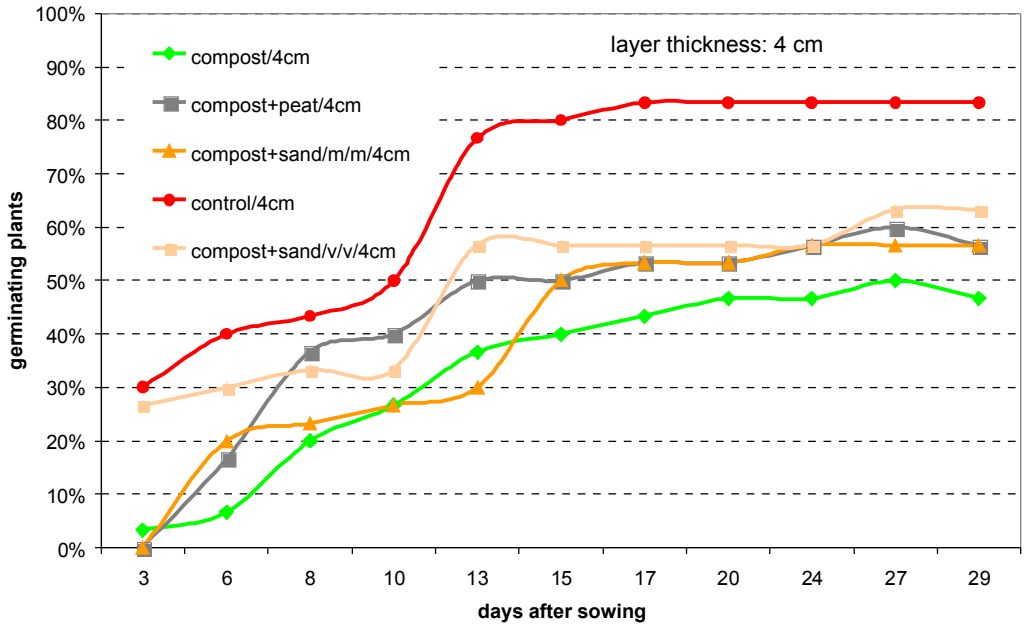

Fig. 5: Germination rate of the three test crops (mean) in compost depending on the dilution material in a 4 cm layer

There was almost no difference between the trays filled with 2 and 4 cm layers. In pure compost only 40 to 50 % of the seeds germinated as compared to the control substrate. Sprouting in pure compost was delayed about ten days. Dilution of compost with peat and sand enhanced the germination of the test plants up to 60%. Figure 6 shows the germination in the control substrate mixed with the digestate, Figure 7 the germination in soil.

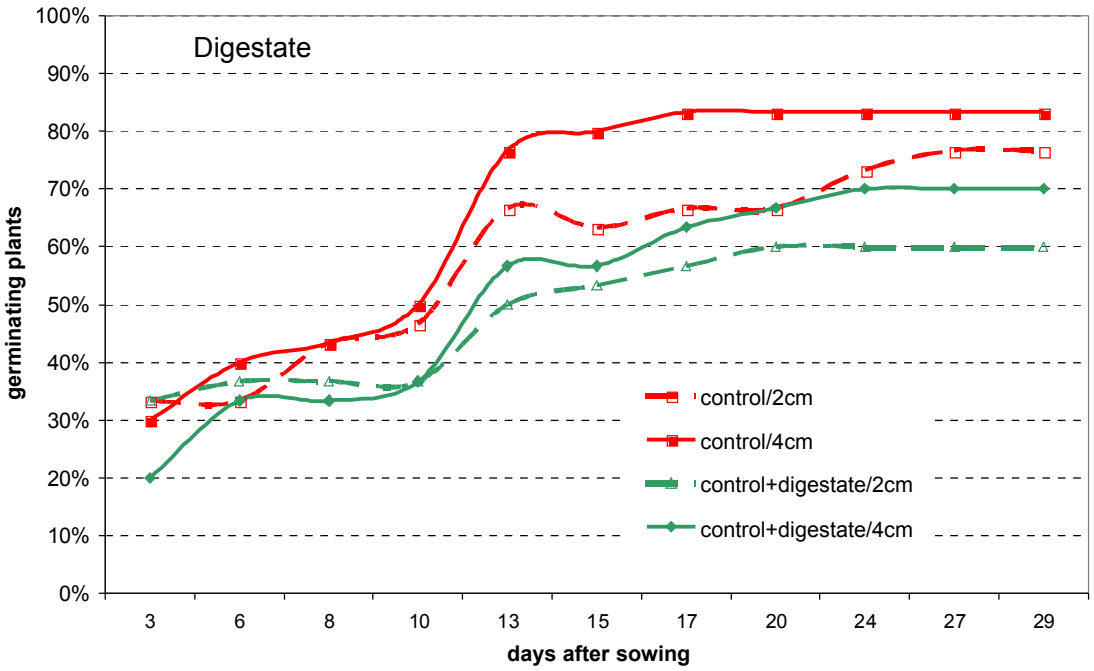

Fig. 6: Germination rate of the test seeds (mean) in the digestate-substrate mix with 2 and 4 cm layers

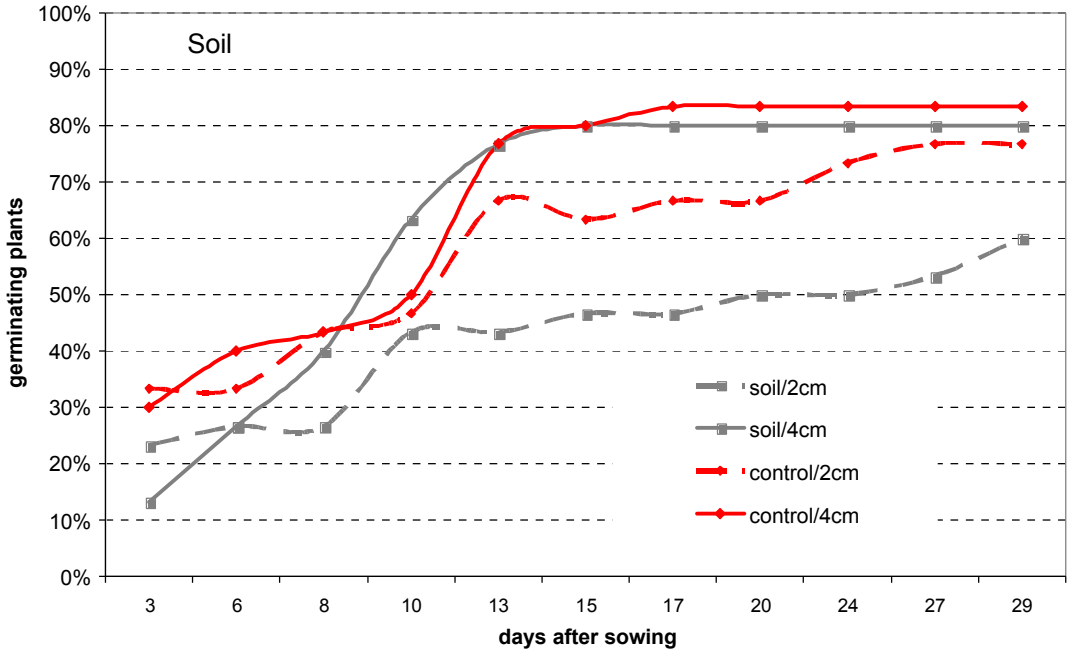

Fig. 7: Germination rate of the test seeds (mean) in soil with 2 and 4 cm layers

In the trays with 4 cm layer of control substrate mixed with the digestate, 70 % of the test seeds germinated. The 2 cm layer showed a slight reduction of germination rate. The pure control substrate led to a germination rate of 80 % in both cases. For soil, the reduction of the layer thickness led to a reduction of the germination of about 20 %.

3.4. Conclusions

- Dilution of compost with peat and sand led to the intended improvement of the germination. No difference between sand and peat as a dilution material was detectable.
- The germination rate in the control substrate – digestate mix was comparable to the diluted compost.
- Layer thickness (2 and 4 cm) showed no influence on the germination rate with the exception of soil, where a reduction of germination with increasing thickness was stated.
- After 24 to 27 days, the germination of Cress, Raygrass and Meldweed was completed.

4. Institut für Gartenbau, Fachhochschule Weihenstephan: Study on the assessment of contamination with viable weeds and plant propagules

4.1. Introduction

The scope of this study was to investigate the influence of the layer thickness and the dilution of materials rich in salt with peat and perlite. Furthermore, the optimal duration of the test was established based on the results of all experiments.

4.2. Experiment 1 – layer thickness, duration of the test

Seeds 11 different species (see table 3) were sown on a peat based growing medium (peat based growing medium - PBGM: weakly decomposed raised bog peat mixed with 6g · L^{-1} $CaCO_3$, 1g · L^{-1} PG-Mix fertilizer: 14 + 16 + 18 and 0,27g · L^{-1} NH_4NO_3) and a mineral soil derived from the experimental site in Freising. Characteristics are given in table 4.

Table 3: Weeds used in the experiment

Name	
botanisch	deutsch
Agrostis capillaris	Rotes Straußgras
Agropyron repens	Quecke
Amaranthus retroflexus	Fuchsschwanz
Capsella bursa-pastoris	Hirtentäschelkraut
Chenopodium album	Gänsefuß
Epilobium angustifolium	Weidenröschen
Plantago major	Wegereich
Poa annua	Rispengras
Rumex acetosella	Sauerampfer
Stellaria media	Sternmiere
Juncus effusus	Binse

Table 4: Characteristics of the tested substrates

	Weakly decomposed raised bog peat	soil
Bulk density (g · L^{-1})	270	1110
pH (H_2O)	6,2	7,4
EC (mS · m^{-1})	26	21
N (CAT, mg · L^{-1})	95	87
P_2O_5 (CAT, mg · L^{-1})	89	90
K_2O (CAT, mg · L^{-1})	118	161

The seeds were placed on a 1 cm layer filled in a plastic tray (dimension 56 x 36.5 x 6 cm, not perforated) and covered by 1, 2, 3, 4 and 5 cm substrate, respectively. The trays were kept in a greenhouse at 22°C/26°C, protected with a foil against further contamination with seeds. The thickness was achieved by calculating the

amount of material needed based on the laboratory compacted bulk density and compressing the material by means of a board to the intended height.

4.3. Results of experiment 1

Only Agrostis capillaris, Agropyron repens, Amaranthus retroflexus, Chenopodium album and Stellaria media showed reasonable germination rates. There was a significant decrease of germination with increasing coverage (figure 8)

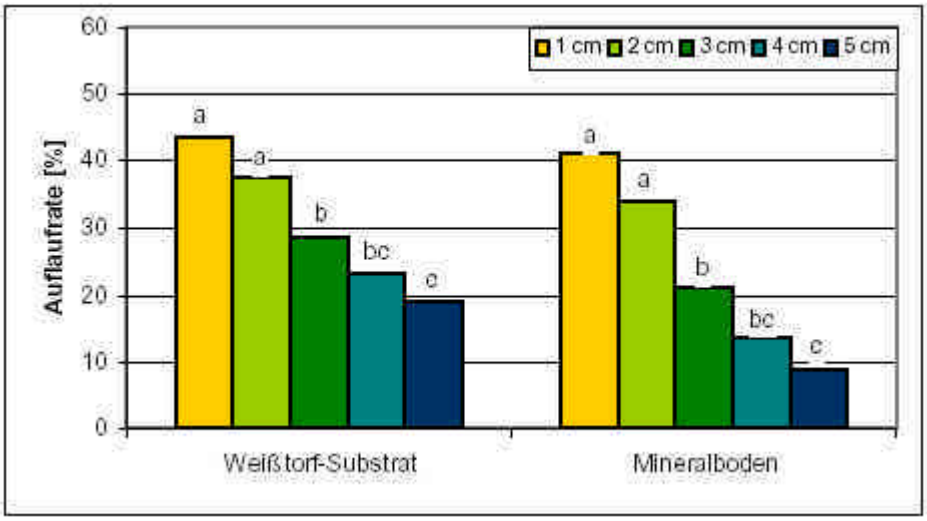

Figure 8: Means of germination rates according to coverage

The differences between individual species are mentioned in the original report. The maximum germination rate was reached after 21 days in most cases (Figure 9), there was no distinct difference between PBGM and soil

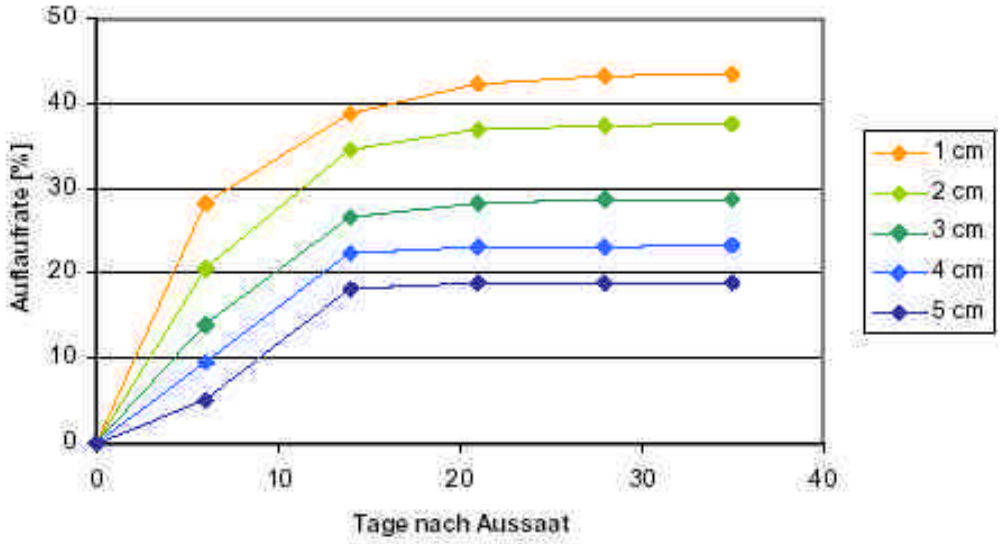

Figure 9: PBGM, Germination rate (%) with time (days after sowing)

4.4. Experiment 2 – Dilution

Two different green waste composts (GGK1, GGK2) were diluted using perlite or weakly decomposed raised bog peat in the ratios 1 + 3, 1 + 1 and 3 + 1 on a v/v

basis. For the 1 + 3 variants, layer of 2 and 4 cm thickness were tested, for the other variants only 2 cm were applied. To simulate high salt content, the composts were mixed with 2,5 and
4,5 g · L^{-1} NaCl, respectively. EC – Values and pH of the substrates are shown in table 5.

Table 5: EC-values and pH of the substrates used

		100 %	+ 25 % Perlite	+ 50 % Perlite	+ 75 % Perlite	+ 25 % peat	+ 50 % peat	+ 75 % peat
GGK 1	EC	194	155	115	72	139	98	43
GGK 2	mS · m^{-1}	262	220	-	-	192	-	-
GGK 1	pH	8,1	8,3	8,5	8,7	7,6	6,9	5,6
GGK 2		8,1	8,4	-	-	7,4	-	-

Seeds were mixed into the material (5 seeds per species and litre), the trays were prepared as in experiment 1. The experiment lasted for 35 days.

4.5. Results of experiment 2

There is a distinct correlation between EC and germination rate (figure 10). There was no significant difference between the 2 and 4 cm layers, although there was a tendency for a reduction with increasing layer thickness.

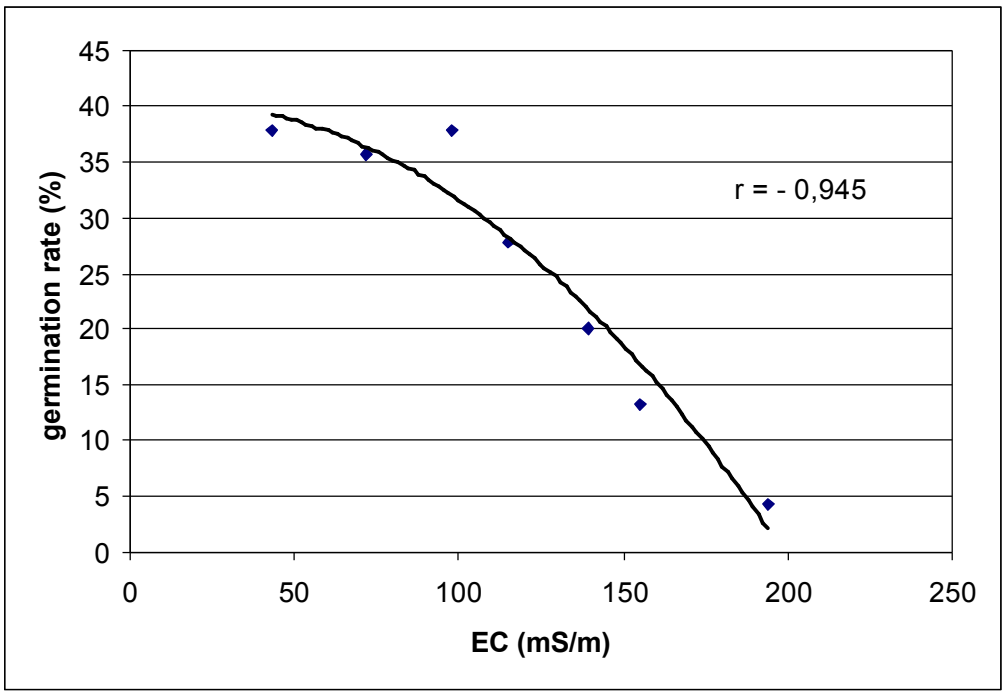

Figure 10: Correlation between germination rate and EC

As a consequence, a dilution of materials high in conductivity is necessary to provide for optimum growing conditions.

4.6. Conclusions

- Although different species react differently to varying layer thickness, 2 – 3 cm layers seem to provide optimum conditions. Especially for materials high in bulk density, 2 cm should not be exceeded.
- The duration of more than 21 days does not improve the results significantly.
- A dilution of the tested material is definitely necessary, if the EC-values exceed 100 $mS \cdot m^{-1}$.
- Peat shall be used as a dilution material.

5. Study by Klasmann-Deilmann GmbH, Gerald Schmilewski: Weed germination in composted biowaste and raised bog peat

5.1. Introduction

The study was focussed on the following factors:

- Material of dilution
- Layer thickness
- pH and EC thresholds
- liming and fertilization requirements
- duration of the test

5.2. Materials and methods

The experiment was carried out using composted biowaste and raised bog peat (H3 – H5). In the following, one litre of material is defined according to EN 13040.

The general setup of the experiment and the used weed seeds are displayed in figure 11 and 12.

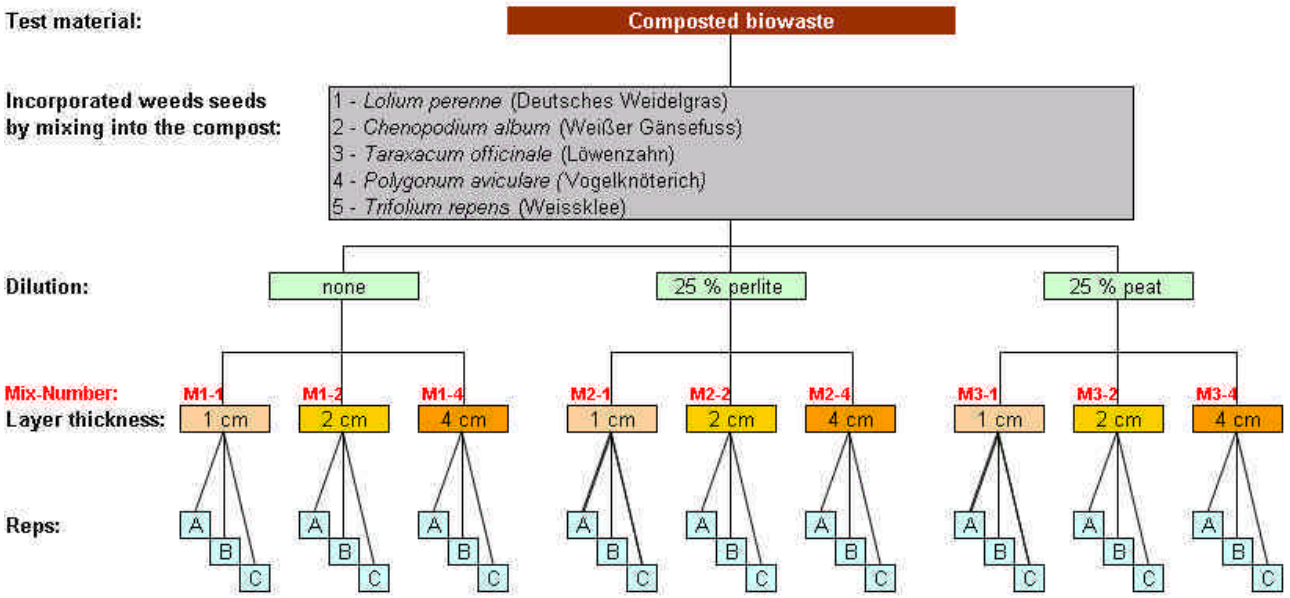

Fig. 11: Design of the experiment using composted biowaste

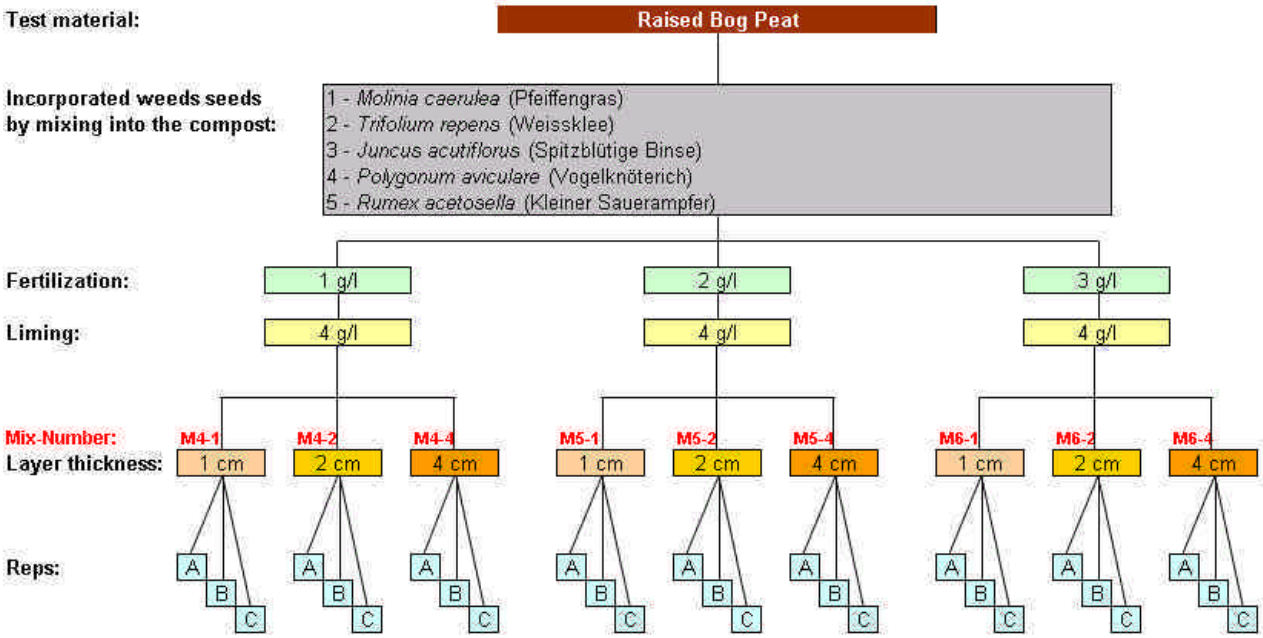

Fig. 12: Design of the experiment using raised bog peat

All materials were analyzed for pH, EC and nutrients according to EN standards. Seeds were obtained from Appels Wilde Samen GmbH in Darmstadt, Germany. Neither the compost nor the diluted compost mixes (M1 to M3) were limed or fertilized, due to high compost pH and EC, the peat had to be limed and fertilized. Standard 60 x 40 cm Piki-Box trays were used for filling. Before filling, seeds were mixed into the test substrates. Sufficient material was then spread into the trays to obtain the given layer thickness. The bottom of the trays was covered with a capillary mat cut to size. This mat was then covered with perforated plastic sheeting. The purpose of this was to reduce drying out of the mixes and to lengthen the intervals between watering, thus reducing work and keeping the mixes at a more regular moisture level. Trays were covered with a fine fleece to avoid air-borne seed contamination. Moistening was carried out by overhead watering, for 6 weeks germinated plants were counted twice a week.

5.3. Results and discussion

Germination (general)

Although purchased from a specialized seed company, not all seed species geminated well and not according to the specifications given by the supplier. Especially in the peat mixes, germination was unsatisfactory with the exception of Trifolium repens. A freeze treatment led to an increase of the germination rate.

Germination and the time factor

After 4 weeks, only a marginal increase of germinating plants could be detected. Longer tests may only be useful for very slowly germinating plant species. This observation was independent from the thickness of the substrate layer.

Germination and the layer thickness

When germination counts are expressed as plants per m^2 of tested material, the thicker layer (4 cm compared with 2 cm; 2 cm compared with 1 cm) will always

provide the higher value as compared to the expression on a volume basis, as more material per m^2 is tested. However, this is not a linear function: the thicker the layer, the less "additional" seeds will germinate, because of the limited ability of the embryos to break through a thicker layer. It is proposed that the expression of results per m^2 or per litre should be optional.

Germination and dilution of test material

Since perlite has a high pH and does not notably decrease the pH of materials with too high pH values, perlite is not recommended. Furthermore, the overall horticultural properties of peat make peat a better diluter than perlite. However, it must be guaranteed that when peat is used as a diluting material, it must be free from weed seeds and other viable propagules.

Germination and EC/salinity of the test material

Materials with a very high EC must be diluted. Fertilizer must be added to materials with a very low EC to ensure more favourable growing conditions for possible weeds. An EC-range between 30 and 50 mS/m seems to be appropriate.

5.4. Conclusions

- Raised bog peat (H3-H5) is recommended as a dilution material.
- The proposed pH range is between 5.5 an 7.0 (EN 13037), it can be obtained by dilution with peat or addition of $CaCO_3$.
- The EC should be in a range between 30 and 50 $mS \cdot m^{-1}$ (EN 13038).
- A layer thickness of 2 cm is suggested. The use a capillary mat covered with a perforated plastic sheet on the bottom of the tray is proposed.
- The duration of the weed test should be 4 weeks.
- To avoid air-borne contamination, the test trays should be covered with a fine fleece material.

6. General conclusions with respect to the final draft:

6.1. Handling of liquid sludge

The handling of the liquid sludge was tested by AGES. Liquid sludges have to be mixed thoroughly with the control substrate in a ratio of 1 + 5 (volume per volume), at least 2000 ml sludge and 10000 ml substrate have to be used.

6.2. Material of dilution

From the results of these experiments it is recommended that weakly decomposed raised bog peat (H3-H5) free of viable weeds and plant propagules be used as the diluent.

6.3. EC threshold

The EC threshold for the dilution is set at 50 mS · m^{-1}.

6.4. Additional treatments

To break seed dormancy, at least one additional treatment seems to be necessary. As the exact fate of the material is not clear at the time of the investigation (landscaping, GM constituent, …), the potential of germinating weeds has to be assessed. Stratification is rather time consuming and also needs proper facilities, so only a treatment with gibberellic acid is proposed. Washing procedures were also effective in reducing the salt content, but in the course of the investigation dilution of the material proved to be sufficient in most of the cases. Only in the case of a phytotoxic effect even after diluting, a washing procedure is proposed.

6.5. Moisture and watering regime

During the experiments it was agreed, that no "sophisticated" description of the watering is necessary. No exact values for water holding capacity or other factors will be mentioned, the formulation as used in the first draft: "The sample has to be kept moist during the whole period by watering in intervals dependent on plant growth and environmental conditions in a practice related manner (good horticultural practice)." seems sufficient.

6.6. Thickness of the layer

The thickness of the layer is set at 2cm.

6.7. Size of test tray

The minimum height of the tray will be set at 50 mm, the dimensions of the tray will be proposed, but only mentioned in an informative way. Perforation will be necessary to provide sufficient drainage and the possibility for subirrigation. Furthermore, the use of a capillary mat covered with a perforated plastic sheet is proposed.

6.8. Duration of the test

The length of the test will be set at 28 days.

Final remark

In the scope of the draft standard, the applicability will be limited to the matrices mentioned in project HORIZONTAL, namely soil, treated biowaste and sludge. It is intended to propose the use of a similar procedure for growing media and soil improvers on the level of CEN (CEN TC 223) as well, however, there will be certain differences, e.g. the necessity for dilution or dormancy breaking procedures and fertilization.

Draft Standard

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 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 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

ISO 10390 Soil Quality – Determination of pH

3 Terms and Definitions

Test sample
Material after sample preparation due to clause 7

4 Principle of the method

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
5.4 Solution of Gibberellic acid ($C_{19}H_{22}O_6$), 1g · L^{-1}
5.5 Calcium carbonate, $CaCO_3$
5.6 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^{-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
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^{-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 the mixing component can be assumed to be between 1 and 4 mS · m^{-1}.

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^{-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 the mixing component can be assumed to be between 1 and 4 mS · m^{-1}.

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^{-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 the mixing component can be assumed to be between 1 and 4 mS · m^{-1}.

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^{-1} 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.

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).

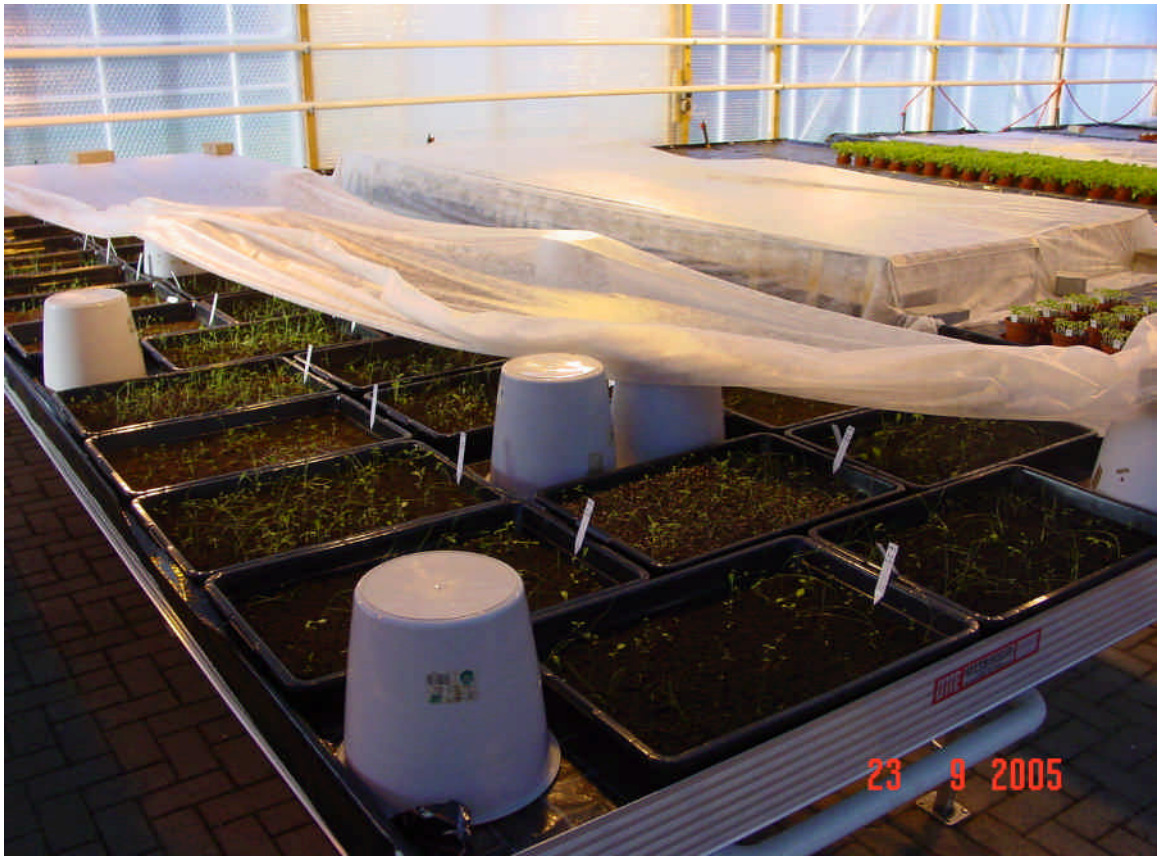

Fig. 1: Cover of the seed trays using a thin fleece

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))

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

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^2 area (equation (2)).

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

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 at the moment

10 Test 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 $CaCO_3$ ($g \cdot L^{-1}$), 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 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

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 (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. Afterwards, the material is dried over night at room temperature. The "washed" biowaste material is then submitted to the test as described above again.

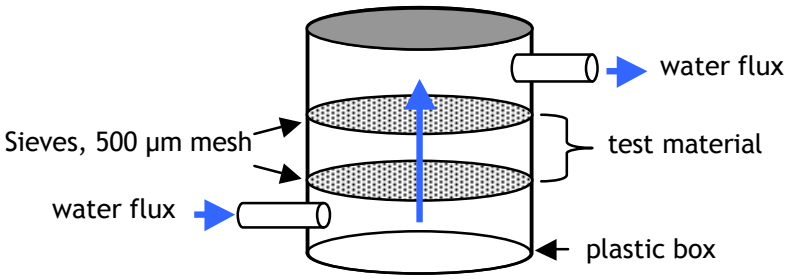

Figure A1: Washing apparatus for performing the washing procedure