Project „HORIZONTAL“ Weeds

Evaluation of the draft method to assess viable weed seeds and plant propagules in soils, sludges and treated biowastes

Introduction

In contrast to usual ring test procedures, it was not possible to use materials with known contents of the substance investigated. As the contamination with seeds is particular, the homogeneity of the distribution of seeds would have been a major problem – both with regard to the production of the test sample and possible de – mixing procedures during the sample transport. So it was decided only to test the ability of the labs to supply conditions suitable for germination of both monocotyledonous and dicotyledonous plants by supplying seeds and giving detailed information about the performance of the test. As it is crucial for the test to optimize pH and EC of the tested sample, the results can be seen in direct relation to the expected precision of the test.

Performance of the test

In preparation of the test, the participating laboratories received 3 different samples (one compost, one soil and one liquid digestate derived from a biogas plant representing sludge), seeds of the species Brassica rapa ssp. pekinensis and Phleum pratense, the method draft and the following information:

Additional measurements required

For a correct performing the test, measurements of pH and EC are requested. For these procedures, use the following standards:

EN 13037 Soil improvers and growing media – Determination of pH
EN 13038 Soil improvers and growing media – Determination of electrical conductivity
ISO 10390 Soil Quality – Determination of pH
ISO 11265 Soil Quality – Determination of the specific electrical conductivity

Pre - treatment procedures

Apart from the procedures referred to in clauses 7 and 8 of the draft standard no additional pre-treatment procedures as described in the informative Annex have to be performed.

NOTE: The performance of these procedures will be demonstrated in a workshop, scheduled for the first half of 2007. The exact time and venue will be announced after finishing the evaluation study.

Adding of the seeds enclosed

For the validation of the test, the enclosed plant seeds have to be added to the test samples by the performing laboratory. Proceed as is described in clause 8.2 control sample.

Control sample

As the germination rate of the enclosed seeds is known, there is no need to include a control sample as described in clause 8.2

Report
Please report the following figures using the attached report template (validation_report.xls):

pH of the test sample
EC of the test sample
Total number of emerged plants
Number of emerged plants per tray
Number of emerged plants in counts per litre, rounded to one decimal place
Average height of the test sample in the tray
Number of emerged plants in counts per m^2, rounded to one decimal place

Results
Results were delivered by 9 laboratories, unfortunately the figures were very inconsistent. For pH and EC determination, not all the laboratories used the prescribed methods, but methods out of the laboratory routine. Whereas pH was comparable at least regarding the dimension, for EC (proposed dimension $mS \cdot m^{-1}$)a comparison was not possible for all the results:

Table 1: pH and EC of the test samples

	pH of the test sample			EC of the test sample		
Lab. Nr.	compost	soil	sludge	compost	soil	sludge
1	6,9	6,9	8	357	22,5	3850
2	7,01	6,95	7,05	51	19	45
3	5,79	6,03	6,93	48	6	9
4	6,2	6,4	6,2	46	7	38
5	6,21	6,37	6,68	41,7	9,5	49
6	6,83	5,94	7	965µS	104 µS	858 µS
7	5,9	6,7	6,1	1631µS	141 µS	1140µS
8	6,63	6,11		2,83mS	0,41mS	
9				47		

From the draft it is clear, that the figure “plants per litre” is regarded to the original substrate used. In the case of the validation study, most of the labs – in accordance with the preparation step of the control sample - added the required 30 seeds to one litre of the final mixture. Finally, there were obviously two types of figures:
number of seeds per litre of final mixture or
number of seeds per litre of original material
In the latter case, numbers were also not consistent because the amount of original sample used differed from lab to lab. Therefore, it was not possible to compare these figures directly. The same applies for the figure “plants per square meter”, as it is calculated from the plants per litre substrate.
Therefore, for evaluating the method, only the number of totally emerged plants in comparison to the sawn seeds were used. Some of the laboratories reported the whole number of emerged plants, some gave the results for Brassica and Phleum separately. For the final evaluation, the total number was used. Additionally, 5 labs reported the number of germinated “unknown” weeds as well – as there is no reference value (see above), these numbers were not considered in the evaluation.

In two cases, for soil the number of these weeds was included in the result, which leads to an exaggeration of the germination rate. As the amount of indigenous weeds it is not know in these cases, 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 2: germination rate of the added seeds (for soil, in 2 cases the number of indigenous weeds is included)

Lab Nr.	germination (%)		
	compost	soil	sludge
1	77,5	83,3	17,4
2	87,5	84,8	88,6
3	96,7	105,0	0,0
4	92,6	92,2	81,0
5	34,0	138,0	1,3
6	93,3	100,0	95,0
7	83,3	80,0	78,9
8	100,0	0,0	n.r.
9	98,7	164,0	100,0

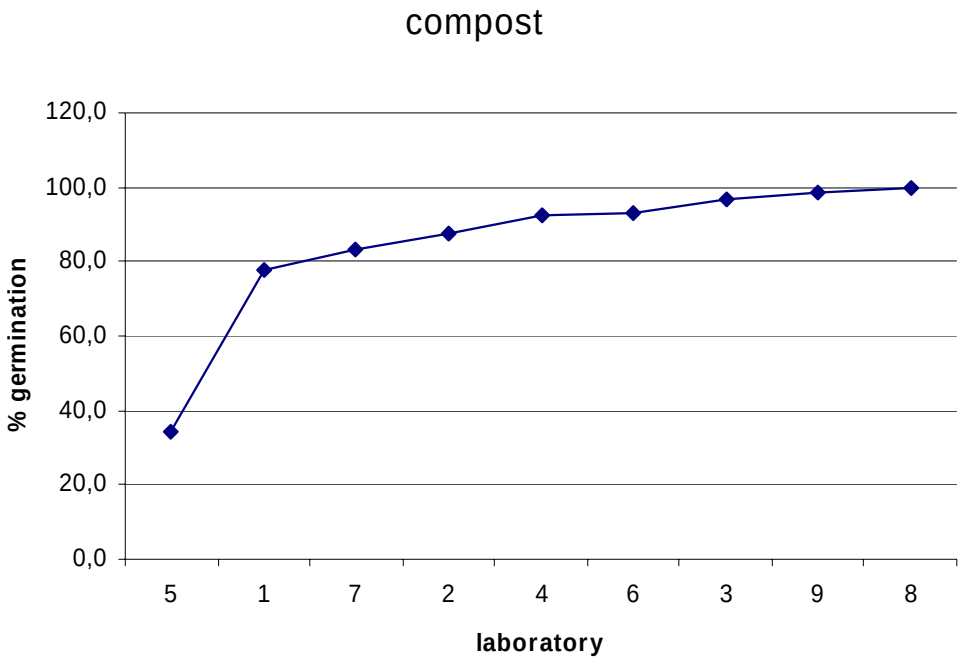

Figure 1: germination rate of seeds in compost

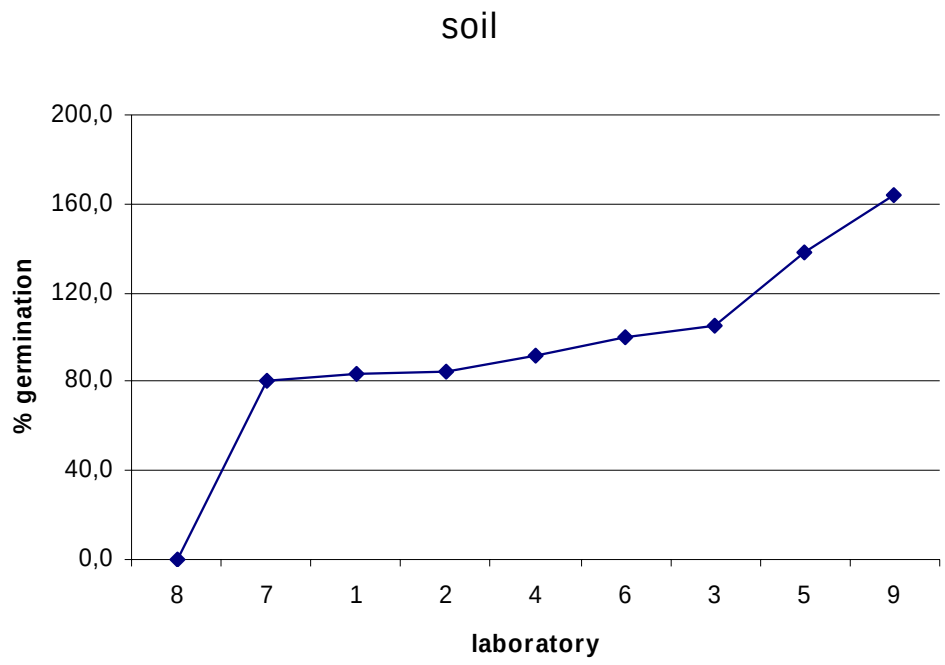

Figure 2: germination rate of seeds in soil

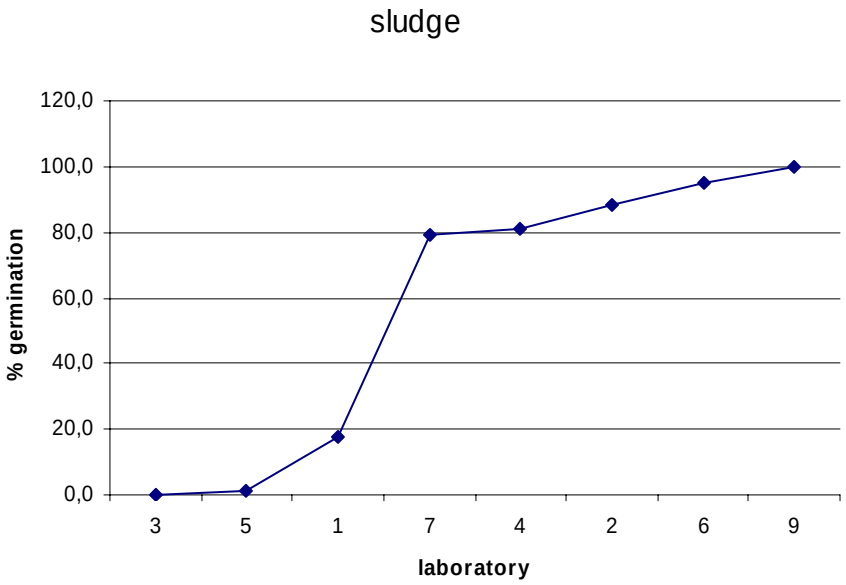

Figure 3: germination rate of seeds in sludge

Repeatability, reproducibility – calculation process

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, 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. As the values were calculated with respect to the ideal value of 100%, it is not sure whether the calculation procedure really is applicable. This should be mentioned in the report. Finally, the amount of labs performing the test in a way that the results can be estimated as valid, seems to be a more valuable figure (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.

Table 3: Repeatability, Repeatability limits, reproducibility, reproducibility limits (%)

	compost	soil	sludge
s_r	8,6	2,5	4,8
r	24,0	7,0	13,6
s_R	8,8	12,8	11,8
R	24,7	36,0	32,9