FINAL REPORT

CRESS TEST

ON

1812/93224

STUDY MST-4/2

SYMPHONY Environmental Ltd.
Elstree House
Elstree Way, Borehamwood
Hertfordshire, WD6 1LE
UNITED KINGDOM

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1. IDENTIFICATION OF TEST

1.1. GENERAL INFORMATION

Project Number
MST-4/2

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Test Item
Blank compost with 0.28% 1812/93224

Reference item
Mature compost without any addition (= blank compost).

1.2. STUDY PERSONNEL

Study Director:	Steven VERSTICHEL
Replacement Study Director:	Eva DE WAEL
Quality Assurance Manager:	Isabella WIERINCK

1.3. STUDY SCHEDULE

Before the actual start-up of the cress test the test item was added to mature compost in a defined concentration and composted at ambient temperatures in the dark for 9 days.

Study initiation date:	Sep-15-04
Study completion date:	Feb-24-05
Experimental starting date:	Sep-15-04
Starting date of composting	Sep-28-04
Completion date of composting	Oct-07-04
Duration of composting	9 days
Starting date of incubation:	Oct-07-04
Completion date of incubation:	Oct-22-04
Duration of incubation:	15 days
Experimental completion date:	Oct-25-04

1.4. ARCHIVING

All raw data and records necessary to reconstruct the study and demonstrate adherence to the study plan will be maintained in the archives of O.W.S. These records include notebooks, study plan, study report, samples of test items and specimens. They will be stored in a file coded:

MST-4/2

The training records of personnel are stored in map ‘Training – GLP’. These files will be kept in the locked ‘Archive Room’, which can only be opened by the archivist or replacement archivist. After ten (10) years, all data and records will be destroyed or returned to the sponsor after agreement in writing by the involved Sponsor and the Study Director. In case no written agreement of the sponsor can be obtained after 10 years, the data and records will be destroyed.

2. CONFIDENTIALITY STATEMENT

The Testing Facility will treat strictly confidential all relevant information on the test items disclosed by the Sponsor as well as all results obtained in executing the Test.

Bruno DE WILDE
Lab Manager

3. GLP COMPLIANCE STATEMENT

The test was performed in accordance with the OECD principles of Good Laboratory Practices (GLP).

Steven VERSTICHEL
Study Director

4. QUALITY ASSURANCE AUDIT STATEMENT

The results reported are in accordance with the Study Plan, SOP's (standard operating procedures) and Raw Data.

An overview of the inspections of the study (with specification of the critical phase) and the auditing and report dates are given in the table below. The results of the inspections are reported to a member of Lab Management Team and Study Director.

Critical phase	Audit date	Report date
At start of incubation (moisture content of test mixtures, preparation of test reactors, selection of seeds)	Oct-07-04	Oct-27-04
At end of incubation (counting, weighing and visual perceptions of the plants)	Oct-22-04	Oct-27-04
After completion of report	Mar-08-05	Mar-08-05

This signed and dated QA Statement ensures that the final report is complete and accurately reflects the conduct and raw data of the study.

Isabella WIERINCK
QA Manager

5. SUMMARY AND CONCLUSIONS

A cress test, which is representative for dicotylenonous plants, was performed on 1812/93224 compost. Test item 1812/93224 was tested for a maximum concentration in a compostable material of 1%. This maximum concentration and the assumption that the compostable material is added to the raw biowaste in a 10% concentration at start of the composting test and is degraded for 90% at the end of the composting test, results in a concentration in the final compost of 0.28% for 1812/93224. The test compost was prepared by mixing the test item in an exact amount with mature compost.

Previous to the plant test a small stabilization process was performed during 9 days in the dark at ambient temperatures in order to prevent possible toxic effects because of biological activity and immaturity of the compost due to the addition of the test item. According to EN 13432 such a stabilization could be continued for 12 weeks. The shortening of the stabilization period does make the exotoxicity test more stringent.

The test is executed according to CEN norm EN 13432 of September 2000 *"Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging"*

No negative effect was seen on the germination and growth of cress in 1/3 and 1/1 test compost/reference substrate mixtures compared to the mixtures of blank compost.

According to CEN norm EN 13432 the germination rate and the plant biomass of the test compost should be more than 90% of those from the corresponding blank compost. This pass value was easily reached for both mixtures of test compost.

In conclusion, it can be stated that the addition to a compostable material of 1812/93224 in a 1% concentration will exert no negative effect on the emergence and growth of cress.

6. INTRODUCTION

6.1. PRINCIPLE OF TEST METHOD

The cress test is used to determine possible toxic effect of a test item to the emergence and growth of terrestrial plants. In this case, the test item is directly mixed with compost and the growth of cress plants in this compost is compared with the growth in blank compost to which no test item is added. The cress plant is chosen as a representative for dicotyledonous plants and because of its sensitive germination.

The test includes germination and growth of cress in mixtures of reference substrate and compost. At the end of the test the fresh and dry weight of the plants is determined for each test series and compared. Also the germination rate is measured. In case there is no significant difference between the test compost and the blank compost, the test item residuals can considered to be non-toxic to plants. The germination rate and the plant biomass of the test compost should be more than 90% of those from the corresponding blank compost.

6.2. GUIDELINES USED

The test is executed according to CEN norm EN 13432 of September 2000 *"Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging"*.

7. MATERIALS AND METHODS

7.1. TEST ITEM

Name:	1812/93224
Physical form:	Granules (diameter ± 2 mm); the granules were ground and the < 1.0 mm fraction was added to compost
Color:	Beige
Code:	BD 92522
Batch number:	8641
Production date:	September 23 2004

7.2. TEST COMPOSTS

Following types of composts were prepared and shortly composted:

Blank compost: < 10 mm fraction of mature compost

Test compost: < 10 mm fraction of the blank compost + 0.28% 1812/93224

7.3. GENERAL PROCEDURE

The cress test is performed in flower pots of 500 ml, containing a mixture of compost and reference substrate. Each compost is tested in 2 mixing ratios of compost and reference substrate: 1/3 and 1/1 on a volumetric basis. Each mixture is tested in 4 replicates. Also the pure reference substrate is tested in 4 replicates.

At the start of the test, each flower pot is filled with 500 ml of compost/reference substrate mixture or reference substrate and 100 ml demi water is added. Subsequently, 100 cress seeds are put on top of the mixture and covered with a thin layer of siliceous sand. Finally, an extra amount of demineralized water can be added to assure optimal moisture content.

After the flower pots have been completely prepared, they are covered with a glass plate and incubated at a constant temperature of 20 ± 2 °C in the dark. After germination, the plate is removed and the pots are exposed to a light intensity of at least 3000 lux during at least 12 hours per day. During the test, extra water is added if needed, and visual perceptions are noted. In order to avoid side effects, the position of each pot is changed 2 times during the testing period, according to a logical rotation scheme.

The test is finished 14 days (± 2 days) after 50% of the control seedlings have emerged. At the end of the test the total fresh and dry weight of the above-soil plant material is determined for each flower pot separately. Also the germination rate is measured.

The toxicity of possible residuals of the test item is evaluated by comparing the results on germination and plant yield of test compost to blank compost. More details on the procedure for the particular test reported, are given in the Study Plan.

7.4. ANALYTICAL METHODS

Except for the weight determination, all analyses are done in duplicate, unless otherwise stated.

Weight Determination

During the test three balances were used. A Sartorius AC 210 S with internal calibration (max. 200 g; d = 0.1 mg) for weighing of plants. A Shimadzu LIBROR EB-3200 D (max. 3120.0 g, d = 0.1 g; max. 600.00 g, d = 0.01 g) was used for the addition of the test items to the compost. A Mettler PJ12 (max. 12000 g, d = 0,1 g) was used for weighing of the composts and reference substrate.

Volumetric Density

The volumetric density is determined by filling a 1 l cylinder and measuring the weight after compression with a 650 g plunger for 180 s. This is repeated three times. The exact procedure is described in “Sop on determination of volumetric density, Edition 6”.

Germinative Capacity

Five ml of demineralized water is added to a petri dish with a filter paper on top of a layer of cotton. Twenty seeds of cress are put on top of the filter paper. A second wet filter paper is put on top of the seeds. The petri dish is sealed with parafilm and left in the dark and at room temperature. After 4 days the number of germinated seeds is counted. The germination is given in % on the amount of seeds at start. The germinative capacity is tested in 5 replicates.

8. RESULTS

8.1. TEST CONDITIONS AND SET-UP

The test compost was prepared by mixing an exact amount of test item 1812/93224 with mature compost. The concentration is based on a maximum concentration of 1% of the test item in the compostable material at start of the composting process and on the assumption that the compostable material is added to the raw biowaste in a 10% concentration at start of the composting cycle. During composting only part of the raw waste and the compostable material will end up in the final compost (fraction < 10 mm), while the test item, in the worst case scenario, will end up completely in the final compost (for more details see Study Plan MST-4/2 amendment 1). The exact composition of the test compost is given in Table 1. The mature compost is the < 10 mm fraction of fresh, pretreated municipal solid waste (biowaste) that has been aerobically composted for more than 20 weeks. The blank and test composts were thoroughly mixed, prior to use.

Because in normal conditions the test item will undergo a composting process, before it comes in contact with plants, a small and simple stabilization process was performed. Therefore the blank and test compost were incubated during 9 days at ambient temperatures in the dark. Without the composting the test item might have a toxic effect to the plants because of biological activity and immaturity of the compost, which is not representative for reality. According to EN 13432 such a stabilization could be continued for 12 weeks. The shortening of the stabilization period does make the ecotoxicity test more stringent.

Table 1. Composition of blank and test compost.

Component	**Blank compost**	**Test compost**
Mature compost (g)	2500.0	2493.1
1812/93224 (g)	-	6.9
% test item in test compost		0.28

In total 20 flower pots were used. The mixtures of reference substrate and compost are given on volumetric basis. In practice, the amounts of compost and reference substrate are added on weight basis, based on measurements of the volumetric density of the compost and reference substrate. Table 2 describes the test set-up.

The used reference substrate is 'Einheitserde O' (EEO), which contains 70% peat and 30% clay and is produced by the 'Einheitserde Werkverband e.V.' in Germany. The reference substrate for this test was obtained from 'Balster Einheitserdewerk GmbH', Eulenstrasse 53, D-5758 Fröndenberg, Germany.

The seeds are cress seeds type ‘large-leaved’ and are derived from AVEVE, Pantserschipstraat 6, B-9000 Gent, Belgium. The cress seeds were examined for their germinative capacity. The germinative capacity was 95%, which is above the recommended value of 90%.

Table 2. Test set-up cress test.

Treatment	Volume of		Weight of	
	Ref. Sub. (ml/pot)	Compost[(a)] (ml/pot)	Ref. Sub. (g/pot)	Compost[(a)] (g/pot)
4 x Reference substrate	500	0	159	0
4 x Blank compost (1:3)	375	125	120	70
4 x Blank compost (1:1)	250	250	80	141
4 x Test compost (1:3)	375	125	120	69
4 x Test compost (1:1)	250	250	80	137

[(a)] Volumetric density of Reference substrate = 0.319 kg/l
Blank compost = 0.563 kg/l
Test compost = 0.549 kg/l

8.2. RESULTS AND DISCUSSION

The test was stopped 12 days after 50% of the control seedlings have emerged. Table 3 represents the average germination rate of the different test series as a percentage of the total amount of seeds added at start. The relative germination rate is also shown in Figure 1. Table 4 shows the average fresh and dry weight yield (of above-soil plant parts) for each test series, as well as the standard deviation. The results are shown in Figure 2.

For statistical analysis of the results, the 'Anova single factor test' at $P \leq 0.05$ was used. The results are compared with the lowest significant difference procedure of Fisher. The results of the statistical analysis are also shown in Figure 1 and 2 as a letter code on top of each bar. Bars, which have at least one letter in common, are not significantly different from each other at the $P \leq 0.05$ level of significance.

The results in Figure 1 show that:

- the germination of cress in the 1/3 mixture of test compost/reference substrate was not significantly different from the germination in the corresponding mixture of blank compost/reference substrate;
- the germination in the 1/1 mixture of test compost/reference substrate was significantly higher than the germination in the 1/1 mixture of blank compost/reference substrate;
- the germination in the reference substrate was significantly higher than the germination in the 1/1 mixture of blank compost/reference substrate, but not significantly different from the other compost mixtures.

Table 3. Germination rate of cress (%).

Test series	Germination rate (%)	
	AVG	STD
Reference substrate	94.8	3.0
Blank compost 1/3	92.8	2.2
Blank compost 1/1	81.3	3.3
Test compost 1/3	95.8	2.8
Test compost 1/1	95.5	1.3

With AVG = average, STD = standard deviation.

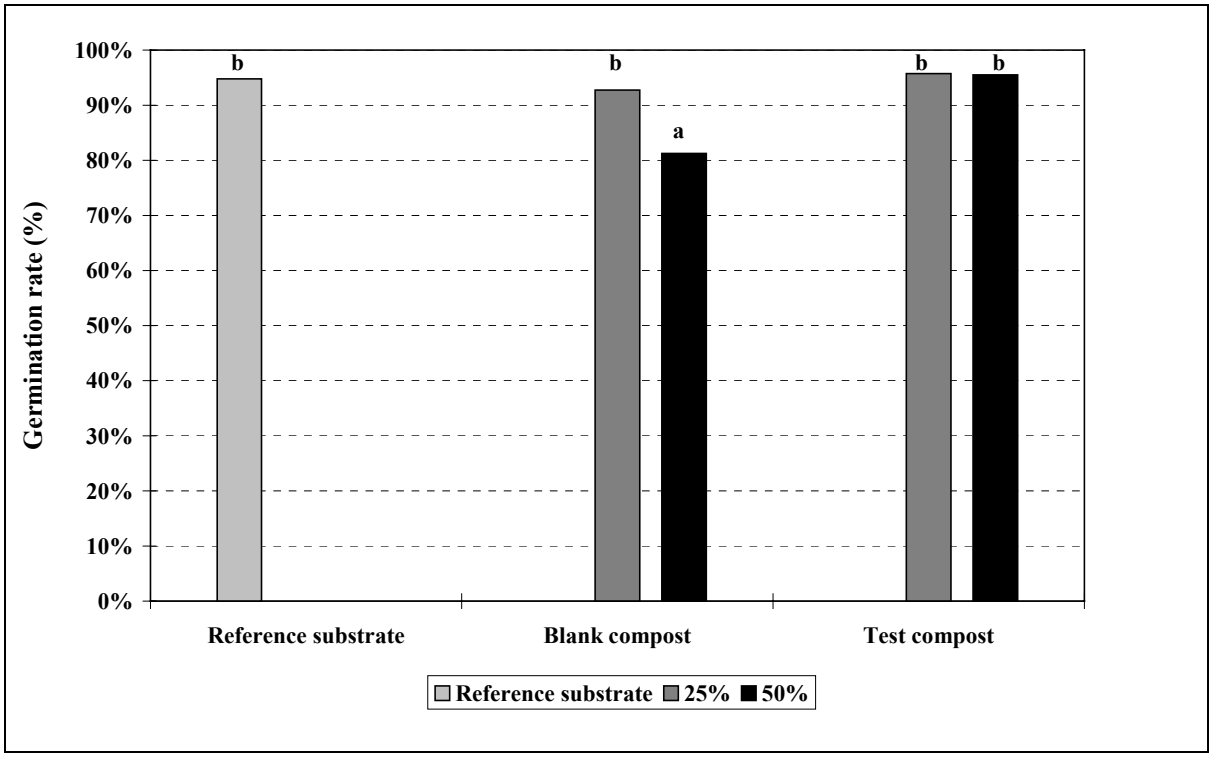

Figure 1. Average germination rate (as percentage of the total amount of seeds added at start). Bars, which have at least one letter in common, are not significantly different from each other at the $P \leq 0.05$ level of significance (Anova single factor Test).

The comparison of plant yield is made on dry weight basis and not on fresh weight because differences in fresh plant yield are not only caused by differences in compost mixtures but also by differences in watering (quantity and timing) and evaporation can have an influence on the fresh weight yield. Dry weight plant yield differences are more directly related to compost and reference substrate conditions. Therefore, dry weight yield is used for further comparison.

From Figure 2 it is seen that:

- the dry weight plant yield of cress in the 1/3 mixture of test compost/reference substrate was not significantly different from the corresponding mixture of blank compost/reference substrate;
- the dry weight plant yield in the 1/1 mixture of test compost/reference substrate was significantly higher than the yield in the 1/1 mixture of blank compost/reference substrate;
- the dry weight plant yield in the reference substrate was not significantly different from the yield in the 1/1 mixture of blank compost/reference substrate, but significantly lower compared to the other compost mixtures.

According to CEN norm EN 13432 the germination rate and the plant biomass of the test compost should be more than 90% of those from the corresponding blank compost. This pass value was easily reached for both mixtures of test compost (see Table 5).

Table 4. Fresh and dry weight yield of cress: absolute and relative to reference substrate.

Test Series	**Fresh Weight Yield**			
	(g)		**(% relative to reference substrate)**	
	AVG	**STD**	**AVG**	**STD**
Reference substrate	2.87	0.23	100	8
Blank compost 1/3	4.96	0.12	173	4
Blank compost 1/1	2.87	0.29	100	10
Test compost 1/3	5.05	0.22	176	8
Test compost 1/1	3.80	0.12	133	4
Test Series	**Dry Weight Yield**			
	(g)		**(% relative to reference substrate)**	
	AVG	**STD**	**AVG**	**STD**
Reference substrate	0.19	0.02	100	10
Blank compost 1/3	0.24	0.01	125	5
Blank compost 1/1	0.20	0.01	106	4
Test compost 1/3	0.25	0.01	130	5
Test compost 1/1	0.24	0.00	125	3

With AVG = average, STD = standard deviation.

Table 5. Germination and dry weight plant yield of both mixtures of test compost 2 as a percentage of the corresponding mixture of blank compost.

Test series	Germination (as % of blank compost)	Dry weight plant yield (as % of blank compost)
Test compost 1/3	103	104
Test compost 1/1	118	118

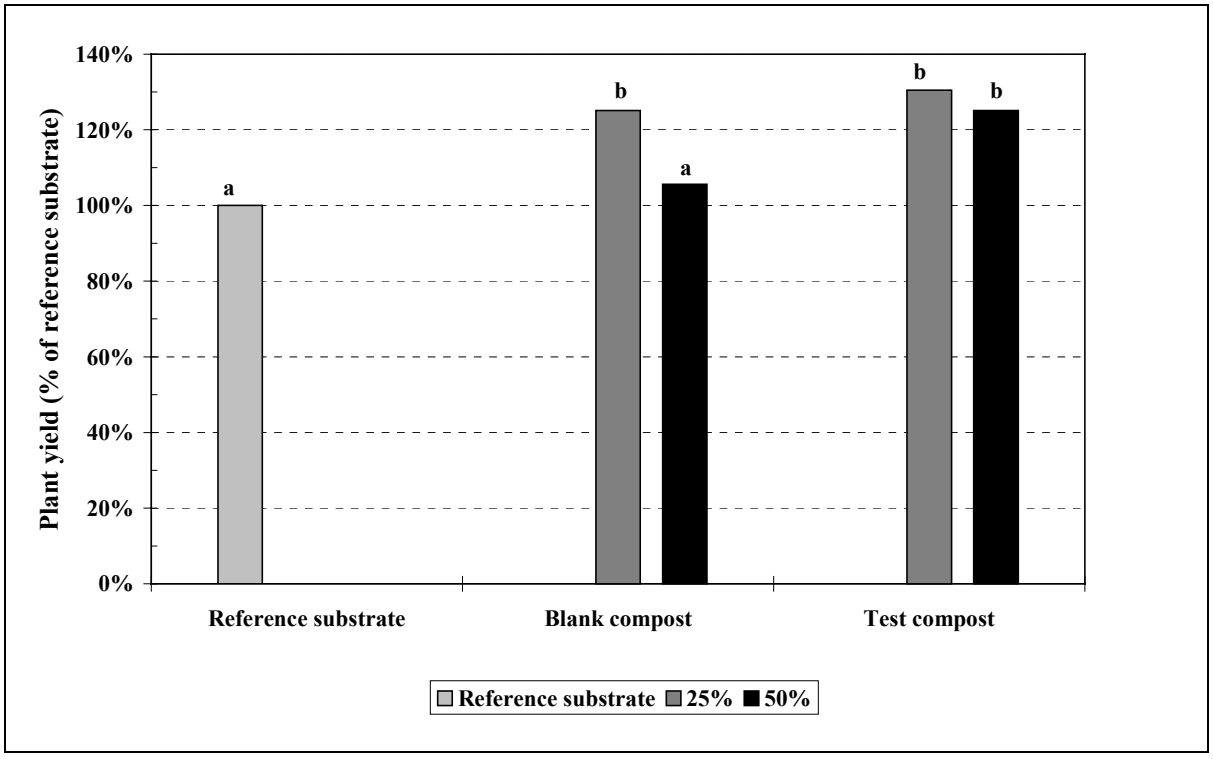

Figure 2. Average dry weight yield of cress (as percentage to reference substrate). Bars, which have at least one letter in common, are not significantly different from each other at the $P \leq 0.05$ level of significance (Anova single factor Test).

Figure 3 and Figure 4 give a visual presentation of the plant growth of cress for the 1/3, respectively 1/1 mixture of compost/reference substrate at the end of the test. In both figures the following test series can be seen from bottom to top: replicates of reference substrate, of the blank compost mixture, of the Sample A compost mixture (see report MST-4/2a), of the Sample B compost mixture (see report MST-4/2b) and of the 1812/93224 compost mixture. The visual observations during and at the end of the test showed that there was a somewhat higher growth in the 1/1 test compost mixture compared to the same mixture of control compost. The 1/3 compost mixtures showed a higher growth compared to the 1/1 mixtures. No signs of chlorosis and necrosis were observed in the compost mixtures. The plants in the reference substrate were smaller and darker.

From the results it can be concluded that the addition to a compostable material of 1812/93224 in a 1% concentration will exert no negative effect on the emergence and growth of cress.

Figure 3. Overview of the cress plants at the end of the test (from bottom to top: reference substrate, control compost 1/3 mixture, Sample A compost 1/3 (see report MST-4/2a), Sample B compost 1/3 (see report MST-4/2b) and 1812/93224 compost 1/3).

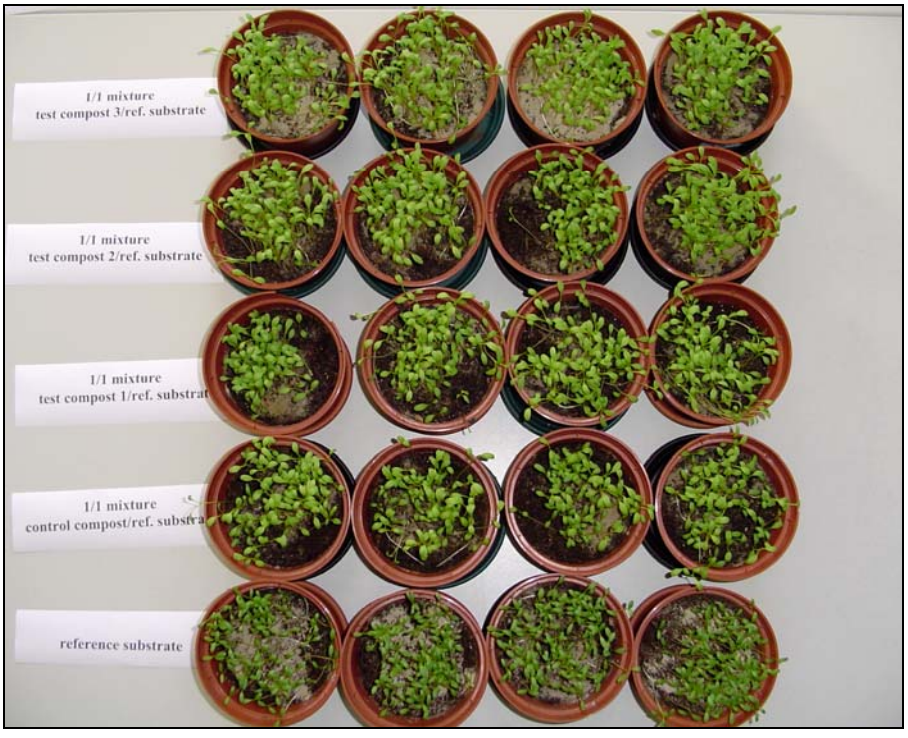

Figure 4. Overview of the cress plants at the end of the test (from bottom to top: reference substrate, control compost 1/1 mixture, Sample A compost 1/1 (see report MST-4/2a), Sample B compost 1/1(see report MST-4/2b) and 1812/93224 compost 1/1).