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ORIGINAL PAPER

Seed germination bioassay for toxicity evaluation of different composting biodegradable materials

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Abstract

In this paper we evaluated the ecotoxicity of the compost obtained after 90 composting days of six different types (compositions or thickness) of the biodegradable materials, using seed germination bioassay. Seeds of 'White Icicle' radish (Raphanus sativus) were sown on filter paper moistened with different concentrations of compost extract (25%, 50%, 75%, 100%) and these were compared with the control (100% distilled water) for each type of material.

After incubation, germinated seeds were counted (G) and root growth (L) measured. Germination index (G_i) was calculated: (G_i) = $G / G_0 \times L / L_0 \times 100$, where G_0 and L_0 are values obtained using distilled water (control).

Taking into account the composition of the materials R1-R7, no direct correlations with that could be found; however it is a clear conclusion that it is a direct correlation of phytotoxicity with the content of the compost extract, all samples being more toxics than the control.

Keywords: ecotoxicity, seed germination, biodegradable materials, composting

Introduction

Composting of new development biodegradable materials is increasing because of environmental toxicity requirements.

Safety requirements have to be met if the produced compost is intended for agriculture use and, until now, the harmfulness of solid waste has been estimated mainly on the basis of its chemical composition [1],[2].

The evaluation of toxicity of composting biodegradable materials by biological testing is therefore extremely important for screening the suitability of waste for agricultural application.

Phytotoxicity has been previously associated with immaturity of compost and reduction of organic acids is correlated with improved plant performance [9].

Phytotoxicity is described as an intoxication of living plants by substances present in the growth medium, when these substances are accumulated in plant tissue [2],[3].

Seed germination and plant growth bioassay are the most common techniques used to evaluate compost phytotoxicity [1]. There are large variations among bioassays and plant species. Fuentes et al.(2004) observed that seed germination has been regarded as a less sensitive method than root length when used as a bioassay for the evaluation of phytotoxicity [4].

According to Kapustka (1997) and Araujo & Monteiro (2005), the seed germination bioassay could be relatively low sensitive to many toxic substances, because many chemicals may not be absorbed by seeds and the embryonic plant draws its nutritional requirements internally from seed stored materials and is effectively isolated from the environment [6],[2].

The roots are responsible for absorption and accumulation of metals so the root lengths were more affected by the concentration of the compost [5],[2].

For the seed germination assay we used the method according to Warman (1999) and Gariglio (2002) [7],[8].

In this study, the toxicity of the compost was evaluated using the germination bioassay for 6 types of biodegradable materials with different composition and/or thickness after 90 composting days. The characteristics of the compost were compared with the control (synthetic solid waste without material after 90 composting days).

Materials and methods

The compost was obtained from synthetic solid waste with a standard composition (Table 1) and was used to preliminary evaluation of the disintegration of packaging materials (samples) with different characteristics (Table 2) under simulated composting conditions in a laboratory scale test, in the seven composting reactors (from 1 to 6 with six types of biodegradable materials and one reactor - no. 7, without material like control).

Table 1. Composition of synthetic solid waste:

Materials	**Dry mass, %**
Sawdust	40
Feeding rabbits	30
Compost	10
Starch	10
Sucrose	4
Corn oil	4
Urea	2
Total	100

Table 2. Characteristics of composite –pressed samples:

No. of composting reactor	**Type of samples/ weight of samples**	**Composition of composite – pressed samples**	**Thickness, mm**
R1.	5.1 /1,720g	BFA40 PLA-Ecoflex (50-50) + 40% WF (La.So.Le) + 5% Lignin (A)	0,121 - 0,147
R2.	5.2/3,785g		0,111 - 0,143
R3.	5.3/3,631g		0,205 – 0,304
R4.	5.4/5,024g		0,223 - 0,317
R5.	7.1/5,083g	BA25 PLA-Ecoflex (50-50) + 25% Lignin (A)	0,249 - 0,334
R6.	8.4/5,392g	BC5 PLA-Ecoflex (50-50) + 5% Lignin (C)	0,142 - 0,248
R7.	Control (without sample)	-	-

Monitoring the disintegration of these packaging materials under simulated composting conditions in a laboratory scale test was according with the EN 14806:2005.

After 90 composting days, we calculated the disintegration rate of the tested materials.

Table 3. The disintegration rate of the tested materials

No. of composting reactor-type of sample	Initial weight, (dry matter), g	Residual weight, (dry matter), g	Disintegration rate, % 100 x (Mi-Mr)/Mi
R1 - M 5.1	1,704	0	100%
R2 - M 5.3	3,611	0,460	87,26%
R3 - M 5.4	5,003	0,167	96,66%
R4 - M 5. 2	3,755	0,038	98,98%
R5 - M 7.1	5,041	0,362	92,81%
R6 - M 8.4	5,382	0,426	92,08%

After 90 composting days we obtained the seven types of compost with the physical and chemical characteristics presented in Table 4:

Table 4. Characteristics of compost after 90 composting days

No. of composting reactor	pH	Organic matter (%)	Volatile Solids* (VS) (%)	Total Dry Solids* (TS) (%)	N_t (%)
R1.	8,8	92	11	37	1,012
R2.	8,9	89	15	36	0,902
R3.	8,4	88	10	37	1,156
R4.	8,6	92	7	38	1,126
R5.	8,7	89	12	36	1,078
R6.	8,9	92	8	38	1,030
R7.	8,5	90	15	35	1,032

* *in accordance with EN 14806:2005*

After the composting process, the phytotoxicity of the composts (R1..R7) was evaluated using the seed germination bioassay according to Gariglio (2002) [1]. In these tests we used radish seeds 'White Icicle' (*Raphanus sativus*).

Germination bioassay

Samples of media were taken after 90 composting days from the seven composting reactors (six with biodegradable materials (R1…R6) and the control (R7) without biodegradable material).

For the extraction process, 100 ml of distilled water were mixed with 50 g media from seven variants of the compost.

The compost – water mixture was shaken for six hours at 25° C, and then centrifuged at 8000 rpm for 20 min at 20° C (Gariglio 2002) [1].

Figure 1. Preparation of compost extracts

The resulted supernatant was diluted with distilled water to yield 0, 25, 50, 75 and 100 % supernatant (extract), thus resulting the samples presented in Table 5.

Table 5. Number of samples resulting after compost dilution

No. of reactor	Sample	Composition of samples	
		extract (ml)	distilled water (ml)
R1	**1.1.** (0% extract)	0	100
	1.2. (25% extract)	25	75
	1.3. (50% extract)	50	50
	1.4. (75% extract)	75	25
	1.5. (100% extract)	100	0
R2	**2.1.** (0% extract)	0	100
	2.2. (25% extract)	25	75
	2.3. (50% extract)	50	50
	2.4. (75% extract)	75	25
	2.5. (100% extract)	100	0
R3	**3.1.** (0% extract)	0	100
	3.2. (25% extract)	25	75
	3.3. (50% extract)	50	50
	3.4. (75% extract)	75	25
	3.5. (100% extract)	100	0
R4	**4.1.** (0% extract)	0	100
	4.2. (25% extract)	25	75
	4.3. (50% extract)	50	50
	4.4. (75% extract)	75	25
	4.5. (100% extract)	100	0
R5	**5.1.** (0% extract)	0	100
	5.2. (25% extract)	25	75
	5.3. (50% extract)	50	50
	5.4. (75% extract)	75	25
	5.5. (100% extract)	100	0
R6	**6.1.** (0% extract)	0	100
	6.2. (25% extract)	25	75
	6.3. (50% extract)	50	50
	6.4. (75% extract)	75	25
	6.5. (100% extract)	100	0
R7	**7.1.** (0% extract)	0	100
	7.2. (25% extract)	25	75
	7.3. (50% extract)	50	50
	7.4. (75% extract)	75	25
	7.5. (100% extract)	100	0

For the germination assay, Petri dishes (10 cm diameter) were lined with fast speed qualitative filter paper. Each dish received 5 ml of the samples obtained according with Table 4 (Sample 1.1., 1.2.,…, 7.7.).

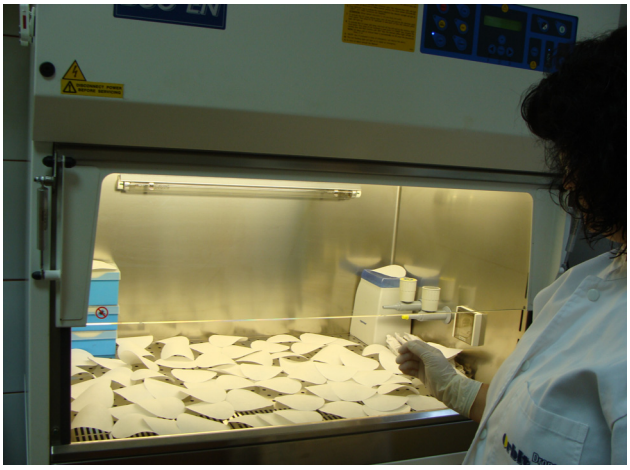

Figure 2. UV sterilization of filter paper for seeds germination samples

Ten 'White Icicle' radish seeds (*Raphanus sativus*) were sown on each disk with two replicates per sample (Petri dish 1 and Petri dish 2).

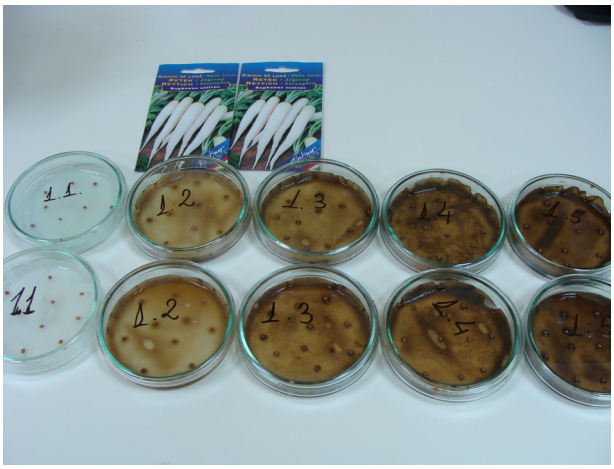

Figure 3. Seeds cultivation in Petri dishes with different compost extract concentrations

After incubation at 25 ^{0}C for 72 hours in the dark, germinated seeds were counted **(G)** and the root length **(L)** was measured. The germination index **(G_i)** was calculated according to the formula $G_i = G/G_0 \times L/L_0 \times 100$, where G_0 and L_0 are respectively the germination percentage and root growth of the 100% dH_2O control. The global germination index **(GI)** was the G_i averages o f the 50 and 75 % extract treatments.

The GI has been proved to be a very sensitive index (Tiquia et al., 1996) indicating, when the values are higher than 80% nonphytotoxicity of the compost [11].

Results and discussion

Composts obtained through biodegradation of the tested materials (see Table 2) inhibited radish seeds germination. All biodegradable materials tested (R1, ..., R6) were more toxic than the control (R7).

In general, the increase of compost concentration affects the radish seeds germination. In control, the seed germination rate was between 95 and 100%.

In the case of R2, R4, R5 and R6, the seeds were germinated at 25% compost extract.

The seed germination rate was 75% for R2 and R4, 95% for R6 and 100% for R5. For higher compost concentration i.e. 50%, 75% and 100%, the seeds germination was inhibited.

Table 6. Germination bioassay results - Number of seeds germinated (G)

No.	Sample	Number of seeds germinated per Petri dish, G		The average number of seeds germinated per sample (G1+G2)/2
		Petri dish 1 (G1)	Petri dish 2 (G2)	
1	**1.1.**	10	9	9,5
2	**1.2.**	0	0	0
3	**1.3.**	0	0	0
4	**1.4.**	0	0	0
5	**1.5.**	0	0	0
6	**2.1.**	10	8	9
7	**2.2.**	7	8	7,5
8	**2.3.**	0	0	0
9	**2.4.**	0	0	0
10	**2.5.**	0	0	0
11	**3.1.**	9	10	9,5
12	**3.2.**	9	8	8,5
13	**3.3.**	10	7	8,5
14	**3.4.**	1	2	1,5
15	**3.5.**	0	0	0
16	**4.1.**	10	9	9,5
17	**4.2.**	9	6	7,5
18	**4.3.**	0	0	0
19	**4.4.**	0	0	0
20	**4.5.**	0	0	0
21	**5.1.**	9	10	9,5
22	**5.2.**	10	10	10
23	**5.3.**	0	0	0
24	**5.4.**	0	0	0
25	**5.5.**	0	0	0
26	**6.1.**	10	9	9,5
27	**6.2.**	9	10	9,5
28	**6.3.**	0	0	0
29	**6.4.**	0	0	0
30	**6.5.**	0	0	0
31	**7.1.**	10	10	10
32	**7.2.**	9	10	9,5
33	**7.3.**	8	9	8,5
34	**7.4.**	7	5	6
35	**7.5.**	3	1	2

Table 7. Germination bioassay results - Root length (L, cm)

No.	Sample	Average root length per Petri dish, L		Average root length per sample (L1+L2)/2, mm
		Petri dish 1 (L1, mm)	Petri dish 2 (L2, mm)	
1	**1.1.**	55	50	52
2	**1.2.**	0	0	0
3	**1.3.**	0	0	0
4	**1.4.**	0	0	0
5	**1.5.**	0	0	0
6	**2.1.**	40	26	33
7	**2.2.**	7	6	6,5
8	**2.3.**	0	0	0
9	**2.4.**	0	0	0
10	**2.5.**	0	0	0
11	**3.1.**	58	46	52
12	**3.2.**	43	54	48,5
13	**3.3.**	25	36	30,5
14	**3.4.**	9	6	7
15	**3.5.**	0	0	0
16	**4.1.**	45	48	46,5
17	**4.2.**	7	5	6
18	**4.3.**	0	0	0
19	**4.4.**	0	0	0
20	**4.5.**	0	0	0
21	**5.1.**	52	45	48,5
22	**5.2.**	13	21	17
23	**5.3.**	0	0	0
24	**5.4.**	0	0	0
25	**5.5.**	0	0	0
26	**6.1.**	41	70	55,5
27	**6.2.**	18	20	19
28	**6.3.**	0	0	0
29	**6.4.**	0	0	0
30	**6.5.**	0	0	0
31	**7.1.**	52	40	46
32	**7.2.**	27	27	27
33	**7.3.**	28	20	24
34	**7.4.**	13	13	13
35	**7.5.**	6	4	5

Figure 4. Percentage of seed germination of radish for different concentration of compost extract

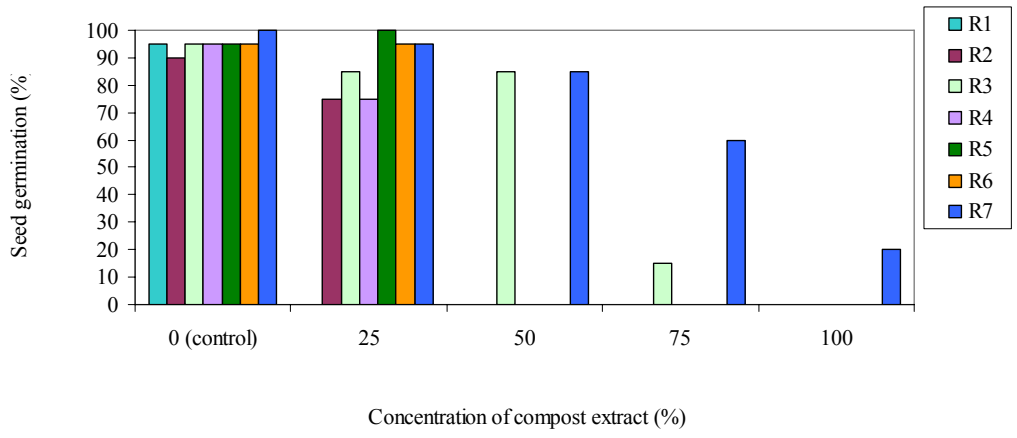

Figure 5. Average root length of samples

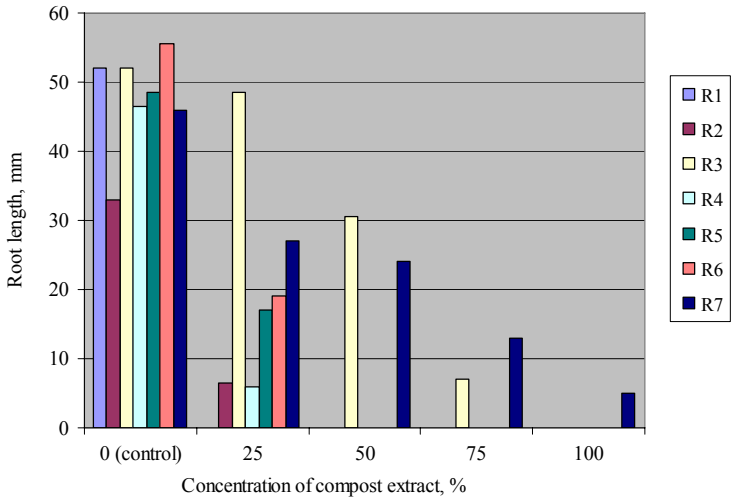

Table 8. Germination bioassay results - Germination index (G_i); Global germination index (GI)

No.	Sample	Germination index (G_i)	Global germination index (GI)
1	**1.1.**	100	0
2	**1.2.**	0	
3	**1.3.**	0	
4	**1.4.**	0	
5	**1.5.**	0	
6	**2.1.**	100	0
7	**2.2.**	15	
8	**2.3.**	0	
9	**2.4.**	0	
10	**2.5.**	0	
11	**3.1.**	100	26
12	**3.2.**	82	
13	**3.3.**	51	
14	**3.4.**	1	
15	**3.5.**	0	
16	**4.1.**	100	0
17	**4.2.**	9	
18	**4.3.**	0	
19	**4.4.**	0	
20	**4.5.**	0	
21	**5.1.**	100	0
22	**5.2.**	36	
23	**5.3.**	0	
24	**5.4.**	0	
25	**5.5.**	0	
26	**6.1.**	100	0
27	**6.2.**	34	
28	**6.3.**	0	
29	**6.4.**	0	
30	**6.5.**	0	
31	**7.1.**	100	31,5
32	**7.2.**	55	
33	**7.3.**	46	
34	**7.4.**	17	
35	**7.5.**	2	

According to GI values, this study proved a higher phytotoxicity of the composts for the all samples including the control (below 80%). The sample R3 demonstrated the lowest phytotoxicity than any other sample.

Conclusions

The phytotoxicity of the compost resulting by composting lab test of the samples with different composition and thickness (Table 2) was studied. Phytotoxicity tests were performed to evaluate the radish seeds germination using different concentrations of the compost extract.

It is found that the phytotoxicity of the studied composts, measured through seeds germination rate and root length is, as follows:

Phyt.R1 > Phyt.R2 = Phyt.R4 > Phyt.R5 = Phyt.R6 > Phyt.R3>Phyt.R7

Taking into account the composition of the materials R1-R7, no direct correlations with that could be found; however it is a clear conclusion that it is a direct correlation of phytotoxicity with the content of the compost extract, all samples being more toxics than the control.

Acknowledgements

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