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*Original Research*

# Ecotoxicity of Composts Containing Aliphatic-Aromatic Copolyesters

**Dana Adamcová[1], Magdalena Daria Vaverková[1]*, Soňa Hermanová[2], Stanislava Voběrková[3]**

[1]Department of Applied and Landscape Ecology, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 61300 Brno, Czech Republic
[2]Department of Polymers, Faculty of Chemical Technology, University of Chemistry and Technology Prague, Technická 5, 16628 Prague, Czech Republic
[3]Institute of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Zemědělská 1, 61300, Czech Republic

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**Abstract**

The eco-toxicological impact of copolyesters during composting was evaluated by plant growth tests with cress (*Lepidium sativum*) and barley (*Hordeum vulgare*). The research was conducted to determine if PET beverage bottles modified by lactic acid (copolyesters) and products of their biodegradation would affect compost. The results demonstrate that the composts on which the examined materials were degrading were not toxic to the plants. Compared to the reference sample, the germination and growth of plants were stimulated. The plants showed an increase in plant biomass. Changes in appearance, retarded growth, or necrotic changes were not observed. The resulting compost did not exhibit any unfavorable influence on compost quality, and the products of degradation affected the growth and development of plants positively.

**Keywords:** biodegradable plastics, compost, phytotoxicity, *Lepidium sativum*, *Hordeum vulgare*

## Introduction

Synthetic polymers are recognized as major solid waste environmental pollutants. Many synthetic polymers, resistant to chemical and physical degradation, are produced and utilized. They present disposal problems when their usefulness ceases. For plastic wastes, an alternative method of disposal is biodegradation [1]. Biodegradation concerns specially designed so-called biodegradable polymers [2]. Increasing amounts of synthetic polymers produce results in increasing interest in polymer biodegradation. The recent incorporation of biological waste treatment in an integrated approach to solid waste management has resulted in a growing commercial interest in the development of biodegradable materials for consumer products [1, 3]. Biodegradable plastics can decompose into carbon dioxide, methane, water, inorganic compounds, or biomass via microbial activities within the natural environment [4]. Biodegradable plastics are designed to degrade under environmental conditions or in municipal and industrial biological waste treatment facilities [5].

Biodegradation of plastics depends on both the environment in which they are placed and the chemical nature of the polymer. There are different mechanisms of polymer biodegradation. The process of polymer biodegradation is affected by many factors [6].

The problem of degradation of biodegradable plastics in environmental conditions has aroused increasing interest, thereby forcing new studies on the behavior of many materials in different environments [7-11].

Composting is one of the oldest methods for processing organic waste, and today this process is more and more

*e-mail: magda.vaverkova@uake.cz

often taken into consideration in waste management strategies [12]. In many European countries the controlled biological treatment of solid waste is considered to be a suitable waste management method. Space for landfill is scarce and therefore expensive in many industrial countries, and biotreatment is a much cheaper alternative. Not only green waste from gardens or biowaste from kitchens can be treated, but any compostable material is in principle suitable, e.g., waste from the food industry or packaging materials made from paper, cardboard, wood, or biodegradable plastics, when not recyclable in other ways. Besides natural materials, synthetic material also can be recycled through composting, if its compatibility with this system is proved [13].

Interest in the ecological effects of composting has been growing recently [13]. Compost is the immediate substrate for cultivated plants, and its properties may limit or stimulate plant development, particularly at the early stages of their growth [12].

International and national quality requirements define that compost shall not contain any environmentally harmful substances. Quality requirements for the compostability of biodegradable materials presuppose that the product does not include any harmful substances or degradation products derived from composted materials. The relative environmental safety of biodegradable plastic materials should be an important consideration during the development stages of these products [13].

Research on ecotoxicity of compost make use of organisms belonging to various taxonomic groups and representing all links of the trophic chain, i.e. bacteria (destructans), plants (producers), and invertebrates (consumers) [13]. Investigations conducted using biotests allow for a general assessment of quality of compost on the basis of a living organism's (bioindicator) response. The response comprises aggregate activities of all substances contained in a given material and shows interactions between them. Such an approach allows us to determine the potential influence of composts on the soil environment. The use of an appropriate set of biotests enables assessment of environmental risks connected with the application of composts prepared from various wastes.

Biological and chemical properties of composts are modified through components used for their preparation and processes occurring during preparation. Especially the use of waste components entails a risk of inappropriate course of the composting process or obtaining a poor-quality product. Therefore, biodegradable polymer wastes introduced into the environment should raise interest. Supporting substances used for their production or compounds formed during the composting process may either inhibit or stimulate plant growth. The degree of hazard may be different and, as in the case of compost salinity, some of the unfavorable effects of such material applications may be eliminated by dissolving them in soil [14]. However, it requires knowledge of not only the level of biodegradability of polymers but also other outcomes of their application, including those resulting from their biological properties.

It is clearly important to study the impact of biodegradable polymers on waste management so as to realize the truth benefit and the need to establish an adequate waste management system and legislation. The research was conducted to determine if PET beverage bottles modified by lactic acid (copolyesters with random microstructure) would affect select biological properties of compost. Research works on the use of biotest set for the estimation of compost toxicity are scarce [12, 15-17]. On the other hand, there are numerous papers dealing with the use of various plant species in estimation of compost phytotoxicity [18-21]. Measurement of seed germination and root growth are the parameters most often used in the assessment of compost toxicity [13]. In the presented investigations the phytotoxicity of compost was estimated by means of a set of biotests using two test organisms. The eco-toxicological impact of model aliphatic-aromatic copolyesters-poly(ethylene terephthalate-co-lactate)s during composting was evaluated by plant growth tests with cress (*Lepidium sativum*) and barley (*Hordeum vulgare*).

## Experimental Procedures

### Materials

Poly(ethylene terephthalate) was obtained from colorless beverage bottles, which were carefully washed and cut into flakes. L-lactic acid (85 % aqueous solution) obtained from Sigma-Aldrich and zinc acetate dihydrate from Fluka AG were used as received.

The investigated materials were prepared according to published procedure [22]. $^{1}$H NMR analysis confirmed the random microstructure of copolyesters prepared. Concretely, four synthesized poly(ethylene terephthalate-*co*-lactate) copolyesters consisted in 41 mol % (sample A), 43 mol % (sample B), 57 mol % (sample C), and 60 mol % (sample D) of aromatic T units, and corresponding aliphatic L units were selected. Films were prepared by pressing of copolyester samples at the temperature of material softening. Square specimens with dimension of 50×50×0.9 mm, sectioned from films, were used for the phytotoxicity experiment.

### Disintegration Tests

The disintegration degree of the copolyester specimens obtained was evaluated following modified version of ČSN EN 14806 Norm "Packaging – Preliminary evaluation of the disintegration of packaging materials under simulated composting conditions in a laboratory scale test" and a modified version of ČSN EN ISO 20200 "Plastics – Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test" (ISO 20200:2004). The materials were mixed with compost and subjected to aerobic degradation. This modification was undertaken in order to bring the test nearer to real conditions.

Table 1. Characteristics of the compost [9].

| Parameters | Value | Unit |
|---|---|---|
| Moisture | 30-65 | % |
| Combustibles | min. 20 | % |
| Total nitrogen | min. 0.6 | % DM* |
| pH | 6.0-8.5 | - |
| Undecomposable ingredients | max. 2.0 | % |
| C:N | max. 30 | - |
| Cd | 2 | mg/kg |
| Pb | 100 | mg/kg |
| Hg | 1 | mg/kg |
| As | 20 | mg/kg |
| Cr | 100 | mg/kg |
| Mo | 20 | mg/kg |
| Ni | 50 | mg/kg |
| Cu | 150 | mg/kg |
| Zn | 600 | mg/kg |

* %DM – % dry matter

For each tested material two reactors were prepared. The authors modified the procedure stated in the Norm and filter paper was used in order to safeguard the presence of suitable conditions for biodegradation. The compost used corresponded to three-month-old mature compost, which was provided by a full-scale aerobic composting plant located in Brno-Černovice (Czech Republic). The characteristics of the compost used are shown in Table 1.

The disintegration experiments were carried out with four types of samples: copolyesters A, B, C, and D. In addition, a reactor containing compost without plastic pieces but with cellulose filter paper was prepared (reference mixture).

The copolyesters and reference materials were used for degradation experiments in the same form as square specimens with dimensions of 50×50×0.9 mm. Copolyester specimens (2.206 g of copolyester A, 3.024 g of copolyester B, 3.099 g of copolyester C and 3.208 g of copolyester D) were mixed with $500 \cdot 10^{-3}$ kg of compost and put into a polypropylene reactor. The polypropylene vessels of 300 mm×200 mm×100 mm (length, width, height) were sealed to avoid excessive evaporation and 5 mm diameter holes at the center of each 100 mm side served for air exchange.

The aerobic degradation was carried out in an air circulation oven (composting bioreactor) at a constant temperature of 58.0ºC (±2ºC). The duration of the incubation was three weeks. During this time, moisture, mixing, and aeration of the samples were periodically controlled. The sample weight loss was calculated as follows:

$$100 \times (m_0\text{-}m_d)/m_0$$

...where $m_0$ means weight of the film before the experiment and $m_d$ means weight of the dried sample after the experiment.

## Water Absorption Test

Previously dried copolyester films were immersed in a Tris-HCl buffer solution (pH 8.50, 0.1 M) and in compost soil at 58ºC for 21 days in the laboratory. For each run, one specimen per tube with 10 ml of Tris-HCl solution or with 43 g of regularly wetted compost soil was maintained. Specimens were periodically removed and dried with filter paper before recording their weight gains. The specimens were reweighed and inserted back into the soil. Experiments were run in triplicate to determine mean values and standard deviations. The relationship used for calculation was as follows:

$$water\ uptake\ (\%) = (m_w - m_0)/m_0 \times 100$$

...where $m_0$ denotes the initial weight of the film, and $m_w$ denotes the weight of the film after exposure to water or soil.

## Plant Material

Seeds used as plant material for testing were commercial seeds of cress (*Lepidium sativum*) and barley (*Hordeum vulgare*). Seeds were surface-sterilized by soaking for 2 min in a commercial sodium hypochlorite (2%) solution with a few drops of Tween-20. Then they were rinsed twice in sterile distilled water.

## Phytotoxicity Test

Phytotoxicity of compost was investigated by means of a set of biotests using two test plants: cress (*Lepidium sativum*) and barley (*Hordeum vulgare*). The possible toxicological effect of soluble degradation products, which were released to the environment during copolyester composting, was assessed according to CSN EN 13432 on the growth of dicotyledonous plants. The medium was specialized soil for germination and plant growth, enriched with compost (25%, 50% w/w). Reference soil was composed of peat and silica sand. Each pot was filled with 200 g of medium, then 100 seeds were placed on the top and covered with a thin layer of silica sand. Plants were grown under controlled conditions for 21 days. Humidity at 70-100% of water absorption capacity, low light intensity, and the laboratory temperature were maintained to be constant. Values obtained from three simultaneously conducted experiments were averaged and presented (germination capacity, plant biomass).

# Results and Discussion

The low water uptake was observed for all tested copolyester samples within the first few days of immersion both in the buffer and in the compost soil (Figs. 1a, b). During this period, the low absorption of water was related to the low rate of its diffusion into a copolyester matrix in a glassy state. The subsequent significant increase measured for copolymers A and B was ascribed to the increase in the

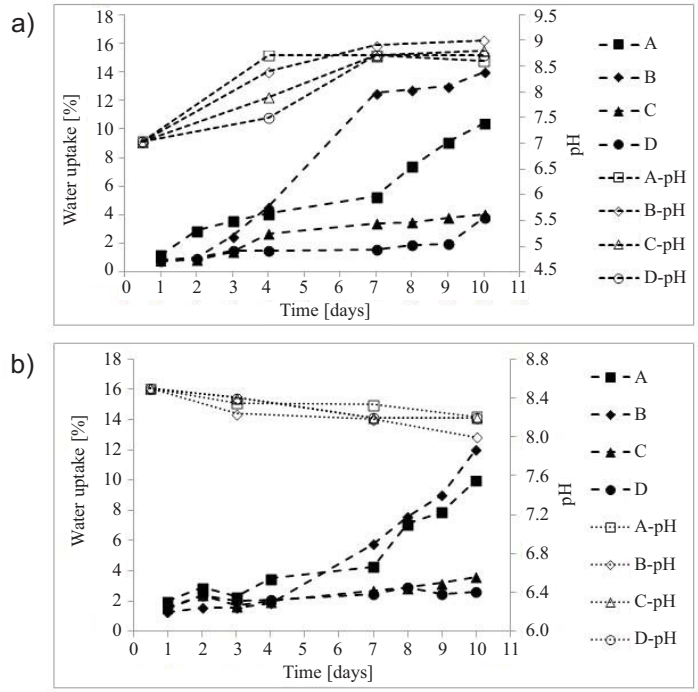


Fig. 1. Weight gained due to water absorption vs pH profile in compost soil (a) and in a Tris-HCl buffer (b); standard error was within 1%.

hydrophilic character of their surfaces due to the generation of hydroxyl and carboxylic groups as products of ester bonds hydrolysis.

The water uptake measured for samples C and D slightly increased over time, which reflects the occurrence of a hydrolytic scission even to a low extent. The development of acidic carboxylic groups was also confirmed by the decrease of pH value from 8.5 to about 8.0 in the Tris-HCl buffer. However, the pH value of compost soil reflected more microbial metabolism changes than the character of degradation products. This was due to its gradual increase from 7.0 to 9.0 during the experiment. Based on these results, copolyester samples with a higher initial content of L units (A, B) were proved to be more susceptible to hydrolytic scission than their counterparts rich in aromatic T units (C, D). The water uptake values of samples were almost the same independent of the incubation environment (soil vs. buffer) and thus the abiotic hydrolysis is supposed to occur dominantly during the initial period of composting.

The photographs (Fig. 2) document the extent of the copolyester specimen's disintegration after 21 days of composting. Hydrolytic scission of ester bonds resulted in the copolyester chains' degradation and loss of specimen integrity.

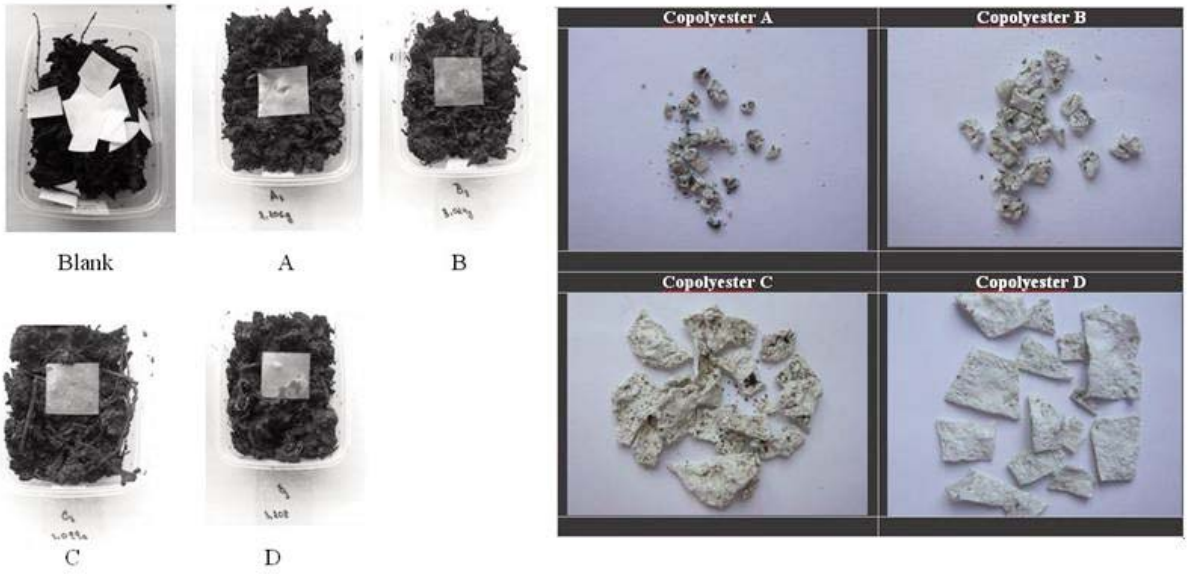


Fig. 2. Examined (composted) samples (A, B, C, and D) and one reference standard at the beginning (left photo) and at the end of the experiment (right photo).

Fig. 3. Layout of the phytotoxicity test.

After the disintegration test the weight losses of copolyesters with prevailing initial L units (A, B) corresponded to each other and were about 90%. The biodegradation rate of copolyesters decreased for sample C (weight loss of 39%) and dramatically for D (weight loss of 5%). These both possessed a dominant portion of aromatic T units with lower accessible ester bonds.

Degradation products released into the surrounding environment are supposed to be based on lactic acid. This was supported by the presence of lactic acid identified in buffer, where copolyesters were abiotically incubated for 21 days.

Phytotoxicity was performed according to instructions given in the standard ČSN EN 13432. Test layout: all dishes were filled with 200 g of the sample (mixture of reference substrate and compost after decomposition of the examined material) and 100 seeds were placed on the surface. The seeds were subsequently covered with a thin layer of inert material (quartz sand) (Fig. 3).

Three parallel determinations were made for each mixture. To prepared samples an amount of water was added to reach 70-100% of moisture-holding capacity. Photographs were taken to document the establishment of the trial (Figs. 3, 4). During the experiment, evaporated water was regularly added as needed. The dishes were kept in a dark place in the laboratory and were covered during the germination period.

Fourteen days after the establishment of the experiment, sprouts and the number of growing plants occurring in the dishes were counted. The data were plotted into tables and

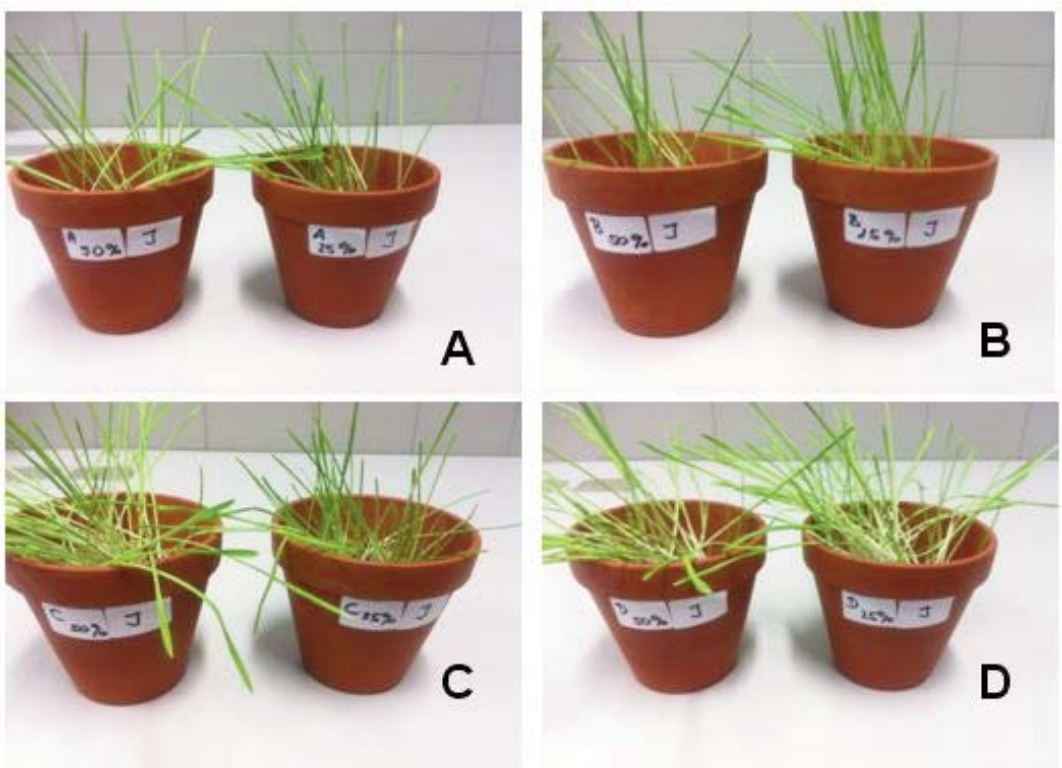


Fig. 4. Samples of *Hordeum vulgare* after 14 days.

Table 2. Germinating capacity of seeds – *Hordeum vulgare*.

| Sample 25% (50 g) | 14 days REF | 21 days REF | Number of seeds sown | Number of seeds germinated | | % of seeds germinated | |
|---|---|---|---|---|---|---|---|
| | | | | 14 days | 21 days | 14 days | 21 days |
| A | 50 | 59 | 100 | 37 | 51 | 74 | 86 |
| B | 50 | 59 | 100 | 45 | 63 | 90 | 107 |
| C | 50 | 59 | 100 | 54 | 85 | 108 | 144 |
| D | 50 | 59 | 100 | 56 | 78 | 112 | 132 |
| Sample 50% (100 g) | 14 days REF | 21 days REF | Number of seeds sown | Number of seeds germinated | | % of seeds germinated | |
| | | | | 14 days | 21 days | 14 days | 21 days |
| A | 50 | 59 | 100 | 35 | 41 | 70 | 69 |
| B | 50 | 59 | 100 | 36 | 44 | 72 | 74 |
| C | 50 | 59 | 100 | 44 | 88 | 88 | 149 |
| D | 50 | 59 | 100 | 46 | 71 | 92 | 120 |

Table 3. Germinating capacity of seeds – *Lepidium sativum*.

| Sample 25% (50 g) | 14 days REF | 21 days REF | Number of seeds sown | Number of seeds germinated | | % of seeds germinated | |
|---|---|---|---|---|---|---|---|
| | | | | 14 days | 21 days | 14 days | 21 days |
| A | 60 | 66 | 100 | 60 | 80 | 100 | 121 |
| B | 60 | 66 | 100 | 48 | 86 | 80 | 130 |
| C | 60 | 66 | 100 | 58 | 95 | 97 | 144 |
| D | 60 | 66 | 100 | 62 | 48 | 103 | 73 |
| Sample 50% (100 g) | 14 days REF | 21 days REF | Number of seeds sown | Number of seeds germinated | | % of seeds germinated | |
| | | | | 14 days | 21 days | 14 days | 21 days |
| A | 60 | 66 | 100 | 85 | 99 | 142 | 150 |
| B | 60 | 66 | 100 | 60 | 90 | 100 | 136 |
| C | 60 | 66 | 100 | 65 | 53 | 108 | 80 |
| D | 60 | 66 | 100 | 62 | 88 | 103 | 133 |

photographs were taken to document the course of the experiment. Germinating capacity and growth of *Hordeum vulgare* is shown in Fig. 4. Twenty-one days from the establishment of the experiment, the counting of sprouts and growing plants was repeated, the results were recorded, and photographs were taken.

The experiment was brought to an end after determination of results, and subsequent establishment of plant biomass. All plant biomass from the individual dishes was removed and weighed. Then it was desiccated in the Ecocell drier at 60ºC, and weighed on analytic digital scales Precisa 4000C; the measured values were recorded.

Results were evaluated from the acquired data. The number of sprouts (number of growing plants) and plant biomass on the compost samples and on the compost from the blank experiment were compared for all mixing ratios. Germinating capacity and plant biomass were calculated as a percentage share of corresponding values obtained from the compost in the blank experiment (see Table 2 – *Hordeum vulgare* and Table 3 – *Lepidium sativum*). Results in the tables (germinating capacity of seeds and plant biomass for the two plant species) are mean values obtained from the conducted experiment results.

The highest germinating capacity of *Hordeum vulgare* seeds (%) with a 25% proportion of compost upon the decomposition of samples after the elapse of 21 days was exhibited by sample C (144%), and the highest germinating capacity of seeds (%) with a 50% proportion of compost after the elapse of 21 days also was shown by sample C (149%).

The highest germinating capacity of *Lepidium sativum* seeds (%) with a 25% proportion of compost upon the decomposition of samples after the elapse of 21 days was

Table 4. Plant biomass – *Hordeum vulgare*.

| Sample 25% (50 g) | Biomass weight REF [g] | Fresh biomass [g] | Fresh biomass against REF % | Dry biomass REF [g] | Dry biomass [g] | Dry biomass % |
|---|---|---|---|---|---|---|
| A | 4.32 | 3.35 | 78 | 0.48 | 0.4 | 83 |
| B | 4.32 | 4.05 | 94 | 0.48 | 0.49 | 103 |
| C | 4.32 | 4.12 | 95 | 0.48 | 0.57 | 119 |
| D | 4.32 | 7.5 | 174 | 0.48 | 0.81 | 169 |
| Sample 50% (100 g) | Biomass weight REF [g] | Fresh biomass [g] | Fresh biomass against REF % | Dry biomass REF [g] | Dry biomass [g] | Dry biomass % |
| A | 4.32 | 3.31 | 77 | 0.48 | 0.39 | 81 |
| B | 4.32 | 2.58 | 60 | 0.48 | 0.34 | 71 |
| C | 4.32 | 8.17 | 189 | 0.48 | 0.84 | 175 |
| D | 4.32 | 6.22 | 144 | 0.48 | 0.63 | 131 |

Table 5. Plant biomass – *Lepidium sativum.*

| Sample 25% (50 g) | Biomass weight REF [g] | Fresh biomass [g] | Fresh biomass against REF % | Dry biomass REF [g] | Dry biomass [g] | Dry biomass % |
|---|---|---|---|---|---|---|
| A | 0.86 | 1.16 | 135 | 0.19 | 0.19 | 100 |
| B | 0.86 | 1.59 | 185 | 0.19 | 0.34 | 180 |
| C | 0.86 | 1.69 | 196 | 0.19 | 0.35 | 184 |
| D | 0.86 | 0.79 | 92 | 0.19 | 0.3 | 158 |
| Sample 50% (100 g) | Biomass weight REF [g] | Fresh biomass [g] | Fresh biomass against REF % | Dry biomass REF [g] | Dry biomass [g] | Dry biomass % |
| A | 0.86 | 1.56 | 181 | 0.19 | 0.28 | 147 |
| B | 0.86 | 1.23 | 143 | 0.19 | 0.29 | 153 |
| C | 0.86 | 1.03 | 120 | 0.19 | 0.3 | 158 |
| D | 0.86 | 1.06 | 123 | 0.19 | 0.26 | 137 |

exhibited by sample C (144%), and the highest germination capacity of seeds (%) with a 50% proportion of compost after the elapse of 21 days was shown by sample A (150%).

Fig. 5 shows the percentage expression of the germination capacity of *Lepidium sativum* (compost shares 25% and 50%) and *Hordeum vulgare* (compost shares 25% and 50%) after 14 days from the beginning of the experiment and after 21 days (end of the experiment).

Data about individual plant biomass weights of *Hordeum vulgare* and *Lepidium sativum* after the elapse of 21 days are summarized in Tables 4 and 5.

The weight of fresh biomass in the 25% and 50% samples of *Hordeum vulgare* ranged from 3.35 to 7.5 g and from 2.58 to 8.17 g, respectively. In the case of dry biomass, the values ranged from 0.4 to 0.81 g in sample 25% from 0.34 to 0.84 g in sample 50% of dry biomass.

The highest value of dry biomass (%) in *Hordeum vulgare* with a 25% proportion of compost upon the decomposition of samples after the elapse of 21 days was exhibited by sample D (169%), and the highest value of dry biomass in *Hordeum vulgare* with a 50% proportion of compost after the decomposition of samples was shown by sample C (175%).

The weight of fresh biomass in the 25% and 50% samples of *Lepidium sativum* ranged from 0.79 to 1.69 g and from 1.03 to 1.56 g, respectively. In the case of dry biomass, the values ranged from 0.19 to 0.35 g in a sample containing 25% and from 0.26 to 0.3 g in a sample containing 50% of dry biomass.

Sample C exhibited the highest values of dry biomass (%) in *Lepidium sativum* with a 25% and 50% proportion of compost upon the decomposition of samples after the elapse of 21 days in both cases (184% and 158%).

Fig. 6 presents a percentage expression of biomass weight for the seeds of *Lepidium sativum* (compost share 25% and 50%) and *Hordeum vulgare* (compost share 25% and 50%) 14 days from the beginning of the experiment and after 21 days (end of the experiment).

## Conclusion

The standard EN 13432:2000: Packaging. Requirements for packaging recoverable through composting and biodegradation stipulates that the examined compost does not exhibit phytotoxicity if the indicator of germinated seeds and the increase of plant biomass are not lower than 90% as compared with plants growing on the control sample. The obtained results clearly demonstrate that the tested copolyesters buried in the compost have no toxic effect on plants. It can be stated that, compared to the reference sample, the germination and growth of plants

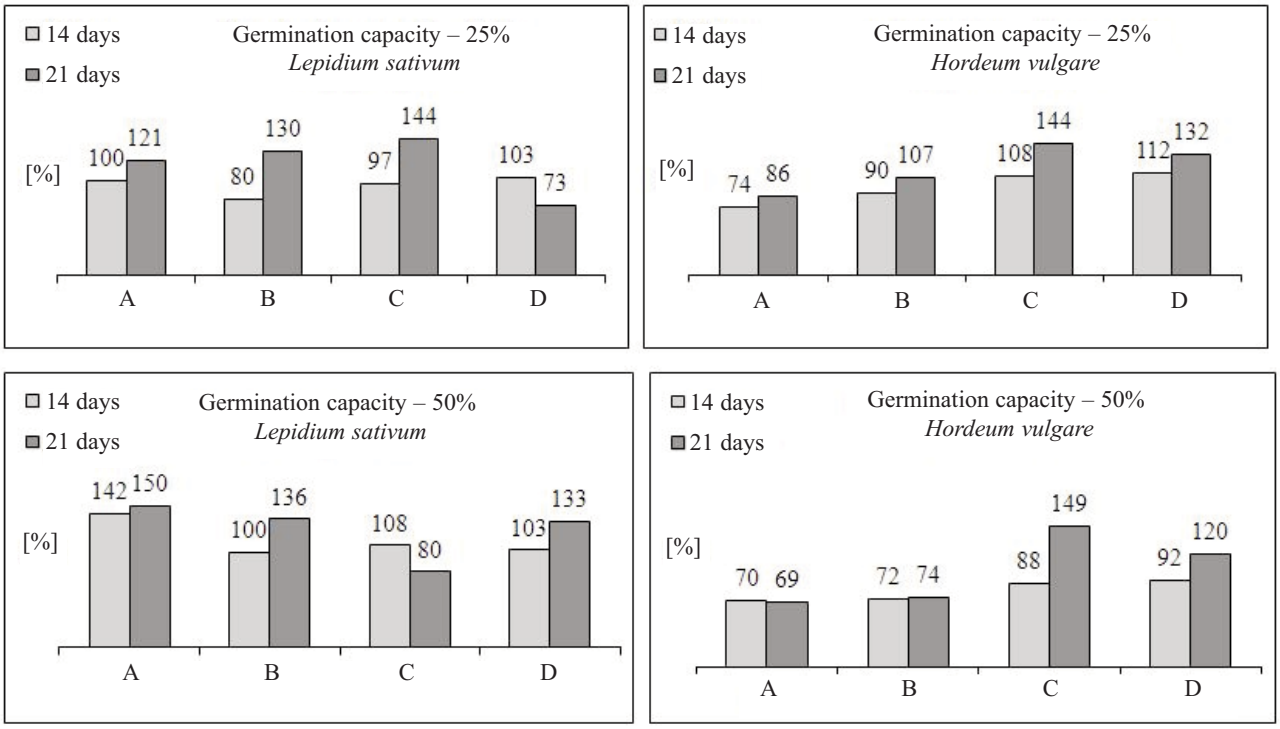


Fig. 5. Comparison of the germination capacity of *Lepidium sativum* and *Hordeum vulgare* seeds.

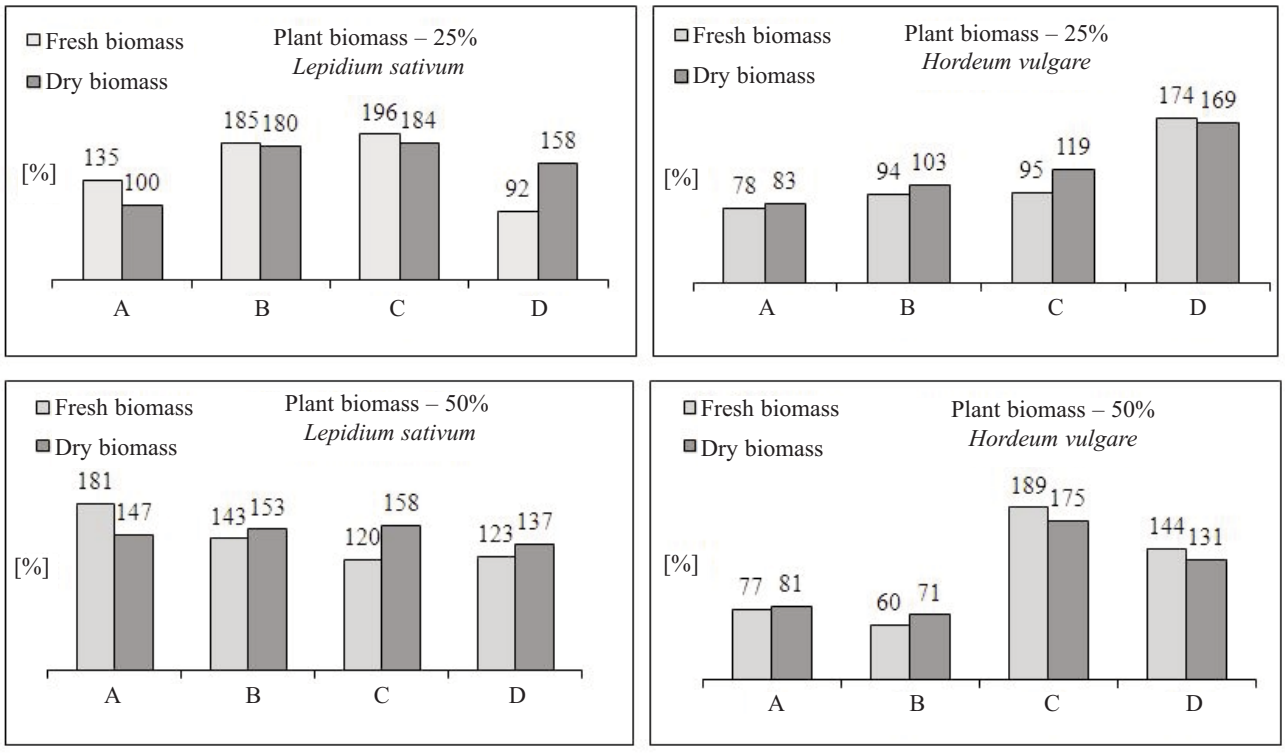


Fig. 6. Comparison of the biomass of *Lepidium sativum* and *Hordeum vulgare*.

were stimulated. The plants growing in the dishes with the compost samples showed an increase in plant biomass. Changes in appearance, retarded growth, or necrotic changes were not recorded.

After the decomposition of the examined materials, the resulting compost did not exhibit any unfavourable influence on the process of composting or compost quality, and the products of degradation affected the growth and development of plants (general biomass increase) positively, too.

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