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SOIL ECOTOXICITY ASSESSMENT AFTER BIODEGRADATION OF SOME POLYMERIC MATERIALS

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Abstract

Plastics are ideal for many applications such as packaging, building materials and different commodities, due to their high durability properties, large availability and lower price. Their widespread use leads to waste disposal problems as plastics degrade in a long period of time, and because of their resistance to microbial degradation, they accumulate in the environment, having a strong negative impact. These facts have led to an increasing interest in the development of biodegradable polymeric materials derived from renewable resources as a method for manufacturing environmentally friendly materials. In order to assess the environmental sustainability, biodegradation studies of PLA/chitosan materials were performed. After the biodegradation process of the tested materials, the soils were analysed in order to evaluate their ecotoxicity using the seed germination bioassay. For this purpose, samples of soil were analysed after 50/100/150 days of biodegradation process and initial soil was used as control. An extraction in water was performed for each sample of soil and the resulted supernatant was diluted to yield 0, 25, 50, 75 and 100% supernatant (extract). The dilutions were then used for seed germination bioassay on radish (Raphanus sativus) and cucumber (Cucumis sativus) seeds. The global germination index (GI), was also calculated. The obtained results showed that the tested soils proved to have a nontoxic effect over the radish and cucumber seeds development, obtaining a GI over 80%.

Key words*: ecotoxicity, seed germination, biodegradabale, polymeric materials.*

INTRODUCTION

Synthetic plastics are the wonder material of today's world, and life without them is unthinkable. Sadly, these same useful qualities are over shadowed by their balanced contribution to rubbish worldwide and its negative outcome for the environment (Imam et al., 2012). Only in Europe, the total production of plastic materials is over 60.000 t per year, and less than 50% of them end up in the official waste stream (Serrano-Ruíz et al., 2018). Therefore, there is a growing need to develop new polymeric materials that are environmentally friendly and can replace petroleum-based ones. Polymers derived from renewable resources present good properties such as availability, compostability, broad and abundant source range, environmentally-friendly and compatibility with foodstuffs and food application (Nur Hanani et al., 2014). Biopolymers represents a growing sector of the plastics market mostly in packaging and disposable products. Biopolymers are plastics that can be manufactured, at least in part, from renewable materials such as corn and sugar cane (Hottle et al., 2017).

Environmental risks from the use of polymeric materials stand in need for the prediction of the life cycle and side effects fastened to them, so the uses of exposure bioindicators/biological indicators are necessary to an integrated risk assessment (Gonçalves et al., 2016). Another required step is to determine the outcomes of the biodegradation processes in the environment, where the material will repeatedly break down and deliver many and different constituents to the soil. It is important to draw attention to the fact that the molecules released from plastic materials may frequently play a role in plant metabolism, or are allelochemicals themselves that balance interactions between plants (Closas et al., 2014). Therefore, biodegradable polymer wastes introduced into the environment should raise interest. Substances used for their development or compounds formed during the degradation process may either inhibit or

stimulate plant growth. Degree of risk may be different and, as in the case of compost salinity, some of the unfavorable effects of such materials application may be eliminated by dissolving them in soil (Kopec et al., 2013).
Seed germination and plant growth bioassay are the most frequent techniques used to evaluate ecotoxicity. In ecotoxicological tests, limit values of a substance or material can be indirect determined by the effects of different concentrations of the substance on the biological variables of individuals from a certain species or community (Lima et al., 2019). A large variety among bioassays and plant species exist. 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 (Mitelut & Popa, 2011).
The purpose of the present study was to determine the ecotoxicity of the soil resulted after the biodegradation of some new polymeric materials, on radish and cucumber seeds.

MATERIALS AND METHODS

Materials
Five polymeric materials were undergone to a biodegradation process in soil according to the standard method SR EN ISO 846/2000, described by Vasile et al. (2018). The materials were composed of PLA (polylactic acid), ATBC (tributyl o-acetyl citrate) or MB (masterbatch) as plasticizers and CH (chitosan). The materials were buried vertically in a glass jar filled with natural soil, characterized by a known capacity to retain water, and with specified water content, which was maintained during the entire experiment. Then, the jars were closed and incubated at a temperature of 25°C for 150 days.
After different periods (50, 100 and 150 days) the resulted soil was tested in order to determine its ecotoxicity on seeds. In Table 1, the soil that was used for analysis was described. The biological material used within these experiments was composed of 2 vegetal species: cucumber seeds (*Cucumis sativus*) and white radish seeds (*Raphanus sativus*).

Table 1. The soil resulted after the biodegradation process of studied biocomposites

No.	Sample of biocomposites undergone to the biodegradation process	Description of analysed soil
1.	-	The control soil, without any samples
2.	**PLA**	The soil in which PLA sample was incubated
3.	**PLA/ATBC**	The soil in which PLA/ATBC sample was incubated
4.	**PLA/MB**	The soil in which PLA/MB sample was incubated
5.	**PLA/ATBC/CH**	The soil in which PLA/ATBC/CH sample was incubated
6.	**PLA/MB/CH**	The soil in which PLA/MB/CH sample was incubated

Working method
For the determination of the ecotoxicity of the soil that resulted after the biodegradation process it was used the method described by Mitelut and Popa (2011).
Thereby, samples of soil were taken after 50, 100 and 150 days of maintaining in soil from the glass recipients, and the soil maintained in the same conditions of temperature (25°C) and humidity without samples buried in it was used as control. For the extraction process, the tested soil was mixed with distilled water, respecting a ratio of 1:2. The soil - water mixture was shaken for 6 hours at 25°C, centrifuged at 8000 rpm for 20 min at 20°C, and then filtered. The resulted supernatant was diluted with distilled water to yield 0, 25, 50, 75 and 100% supernatant (extract).
For seed germination assay, glass Petri dishes (10 cm diameter) were lined with fast speed qualitative filter paper and sterilized. Each dish received 5 ml of extract (10 Petri dishes/sample/concentration/type of seed). In each Petri dish were then distributed 10 seeds of the tested species (cucumber/radish). After incubation at 25°C for 72 hours in the dark, germinated seeds were counted, and the root length was measured.
The germination index (Gi) was calculated according to the formula:

$$\boldsymbol{Gi = \frac{G}{G_0} \times \frac{L}{L_0} \times 100}$$

where G_0 and L_0 represent the germination percentage and rootlet growth of the 100% distilled water control (0% supernatant).
It was also calculated the global germination index (**GI**), which was the Gi averages of 50 and 75% dilution treatments for each sample.

This index represents a very sensitive index which indicates the fact that the soil has no phytotoxic effect when its values are over 80% (Tiquia, 1996).

RESULTS AND DISCUSSIONS

The increase of supernatant concentration inhibits the germination capacity of cucumber seeds (Table 2), when tested after 50 days of incubation. After 150 days of incubation in soil, the germination capacity increased and the highest values were obtained for the soil in which PLA/ATBC/CH sample was incubated. The lowest values of the germination capacity of cucumber seeds was recorded by the control soil, on the entire period of testing.

Table 2. Germination capacity of cucumber seeds (%) (mean values of 100 seeds with standard deviation)

Incubation period (days)	Soil Sample / Supernatant concentration	Control soil	PLA	PLA/ATBC	PLA/MB	PLA/ATBC/CH	PLA/MB/CH
50	25%	88.88 ± 10.54	76.66 ± 20.61	85.55 ± 7.26	93.33 ± 7.07	72.22 ± 13.94	73.33 ± 14.14
	50%	78.88 ± 10.54	85.55 ± 11.30	81.11 ± 16.15	63.33 ± 14.14	75.55 ± 19.43	48.88 ± 14.52
	75%	67.77 ± 25.38	88.88 ± 7.81	77.77 ± 19.22	83.33 ± 7.07	75.55 ± 11.30	54.44 ± 10.13
	100%	72.22 ± 14.81	81.11 ± 15.36	75.55 ± 13.33	67.77 ± 18.55	87.77 ± 6.66	70.00 ± 20.00
100	25%	88.88 ± 10.54	97 ± 6.74	69 ± 21.31	89 ± 11.00	98 ± 4.21	100 ± 0.00
	50%	78.88 ± 10.54	89 ± 14.49	67 ± 20.57	92 ± 7.88	94 ± 4.21	99 ± 3.16
	75%	67.77 ± 25.38	81 ± 21.83	72 ± 28.20	93 ± 8.23	99 ± 3.16	99 ± 3.16
	100%	72.22 ± 14.81	97 ± 6.74	87 ± 14.18	96 ± 6.99	94 ± 8.43	98 ± 4.21
150	25%	88.88 ± 10.54	98 ± 4.21	98 ± 4.21	87 ± 10.59	98 ± 4.21	89 ± 7.37
	50%	78.88 ± 10.54	100 ± 0.00	97 ± 4.83	80 ± 15.63	100 ± 0.00	89 ± 9.94
	75%	67.77 ± 25.38	97 ± 4.83	79 ± 9.94	79 ± 11.00	98 ± 4.21	86 ± 15.05
	100%	72.22 ± 14.81	94 ± 8.43	100 ± 0.00	82 ± 13.16	99 ± 3.16	89 ± 7.37

Regarding the rootlet length of the germinated cucumber seeds (Table 3), it was observed that higher values were obtained for all tested soils compared to the control soil. The values of the rootlet length increased with the increasing period of incubation, meaning that once the polymeric materials start to degrade, the resulted compounds go into the soil and represent good nutrients for plant growth, comparing with the results obtained for control soil (without samples buried it it). These results are in accordance with the results obtained for the germination capacity of cucumber seeds.

Table 3. Rootlet length of cucumber seeds (mm) (mean values of 100 seeds with standard deviation)

Incubation period (days)	Soil Sample / Supernatant concentration	Control soil	PLA	PLA/ATBC	PLA/MB	PLA/ATBC/CH	PLA/MB/CH
50	25%	7.98 ± 6.63	6.69 ± 3.34	12.76 ± 3.16	20.55 ± 4.49	8.56 ± 4.45	15.46 ± 18.79
	50%	3.8 ± 3.26	5.68 ± 3.59	9.1 ± 4.11	13.52 ± 4.54	14.38 ± 7.80	9.17 ± 6.43
	75%	20.58 ± 9.02	6.87 ± 2.19	9.47 ± 4.45	20.43 ± 3.19	16.41 ± 5.77	9.74 ± 3.78
	100%	6.85 ± 4.38	11.86 ± 10.60	9.47 ± 4.45	15.68 ± 5.85	20.22 ± 3.27	8.12 ± 2.24
100	25%	7.98 ± 6.63	39.09 ± 5.02	15.22 ± 7.17	24.73 ± 8.60	41.93 ± 4.86	51.05 ± 2.95
	50%	3.8 ± 3.26	20.53 ± 12.61	10 ± 6.40	25.71 ± 4.47	56.3 ± 7.10	50.51 ± 4.73
	75%	20.58 ± 9.02	39.28 ± 18.79	33.09 ± 20.16	37.69 ± 5.68	51.81 ± 4.52	56.14 ± 5.71
	100%	6.85 ± 4.38	41.92 ± 5.49	24.98 ± 16.22	38.27 ± 9.70	46.84 ± 6.79	55.34 ± 6.40
150	25%	7.98 ± 6.63	58.15 ± 5.75	50.76 ± 4.16	36.52 ± 7.83	47.12 ± 4.87	40.42 ± 3.10
	50%	3.8 ± 3.26	43.45 ± 3.43	54.28 ± 7.92	46.63 ± 6.67	46.55 ± 3.31	33.01 ± 6.19
	75%	20.58 ± 9.02	61.09 ± 5.59	29.59 ± 4.10	47.02 ± 8.21	48.5 ± 2.72	41.63 ± 6.85
	100%	6.85 ± 4.38	58.57 ± 6.80	44.65 ± 5.14	36.11 ± 7.93	45.26 ± 5.09	39.71 ± 5.78

The germination capacity of radish seeds (Table 4) was over 90% for the control soil and for the soil in which PLA/ATBC/CH sample was maintained for all testing periods and concentrations. For the PLA/MB sample for every testing period, for at least one sample the GI was below 80%. However, in general, the values obtained for radish seeds were higher when compared to the germination capacity of cucumber seeds.

Table 4. Germination capacity of radish seeds (%) (mean values of 100 seeds with standard deviation)

Incubation period (days)	Soil Sample / Supernatant concentration	Control soil	PLA	PLA/ATBC	PLA/MB	PLA/ATBC/CH	PLA/MB/CH
50	25%	93 ± 8.23	97 ± 6.47	93 ± 6.74	88 ± 6.32	98 ± 4.21	93 ± 4.83
	50%	93 ± 8.23	97 ± 6.74	91 ± 11.00	90 ± 4.71	97 ± 6.74	92 ± 6.32
	75%	91 ± 7.37	92 ± 7.88	92 ± 6.32	79 ± 8.75	95 ± 7.07	87 ± 8.23
	100%	96 ± 6.99	84 ± 27.16	96 ± 5.16	91 ± 5.67	95 ± 8.49	86 ± 6.99
100	25%	93 ± 8.23	88 ± 9.18	72 ± 12.29	62 ± 35.83	96 ± 5.16	96 ± 6.99
	50%	93 ± 8.23	91 ± 8.75	92 ± 6.32	87 ± 8.23	91 ± 7.37	86 ± 10.74
	75%	91 ± 7.37	90 ± 9.42	83 ± 10.59	69 ± 13.70	96 ± 5.16	95 ± 7.07
	100%	96 ± 6.99	92 ± 7.88	88 ± 10.32	92 ± 9.18	99 ± 3.16	93 ± 8.23
150	25%	93 ± 8.23	90 ± 10.54	96 ± 5.16	81 ± 12.86	93 ± 8.23	86 ± 11.73
	50%	93 ± 8.23	96 ± 5.16	92 ± 7.88	86 ± 9.66	96 ± 6.99	78 ± 17.51
	75%	91 ± 7.37	92 ± 7.88	95 ± 5.27	85 ± 8.49	95 ± 7.07	78 ± 17.51
	100%	96 ± 6.99	94 ± 8.43	88 ± 9.18	75 ± 19.57	93 ± 10.59	85 ± 13.54

The rootlet length of the germinated radish seeds was measured (Table 5). It was observed that the lowest values were obtained for control soil, while the highest values were obtained for the soils in which PLA/ATBC/CH and PLA/MB samples were incubated for 50 days. After 100 days of incubation, a slightly inhibition effect was clearly observed for PLA/MB sample in comparison with the control and the other samples.

Table 5. Rootlet length of radish seeds (mm) (mean values of 100 seeds with standard deviation)

Incubation period (days)	Soil Sample / Supernatant concentration	Control soil	PLA	PLA/ATBC	PLA/MB	PLA/ATBC/CH	PLA/MB/CH
50	25%	28.47 ± 4.12	29.02 ± 5.72	33.78 ± 4.22	66.73 ± 13.81	51.39 ± 10.77	52.7 ± 6.48
	50%	30.04 ± 7.98	30.76 ± 2.88	33.52 ± 7.43	62.92 ± 9.02	50.07 ± 11.62	55.33 ± 7.77
	75%	33.27 ± 5.62	27.58 ± 5.94	29.14 ± 4.24	61.38 ± 11.16	49.29 ± 7.62	61.57 ± 7.06
	100%	31.03 ± 5.71	30.65 ± 4.25	39.8 ± 9.17	70.56 ± 12.79	49.32 ± 8.00	60.88 ± 6.88
100	25%	28.47 ± 4.12	49.7 ± 7.33	30.69 ± 7.63	13.25 ± 11.55	49.26 ± 8.98	40.21 ± 7.22
	50%	30.04 ± 7.98	44.8 ± 8.02	30.5 ± 5.38	41.14 ± 8.42	45.97 ± 6.80	57.12 ± 7.01
	75%	33.27 ± 5.62	53.79 ± 9.55	41.14 ± 10.91	16.94 ± 6.37	41.29 ± 9.04	49.88 ± 10.04
	100%	31.03 ± 5.71	52.28 ± 8.90	37.69 ± 7.78	28.95 ± 6.24	42.55 ± 7.76	51.12 ± 11.53
150	25%	28.47 ± 4.12	38.52 ± 6.85	50.15 ± 9.05	50.06 ± 12.48	57.72 ± 8.79	42.87 ± 8.96
	50%	30.04 ± 7.98	40.39 ± 10.08	44.89 ± 3.63	60.2 ± 9.42	41.22 ± 3.62	39.04 ± 11.06
	75%	33.27 ± 5.62	51.86 ± 8.09	54.86 ± 5.62	56.84 ± 3.98	45.1 ± 8.69	54.79 ± 8.52
	100%	31.03 ± 5.71	41.46 ± 11.05	48.19 ± 7.47	32.94 ± 11.22	54.17 ± 10.64	50.29 ± 10.96

According to GI values (Figures 1 and 2), the tested soils that resulted after the polymeric materials biodegradation tests for 150 days, proved to have a nontoxic effect over the radish and cucumber seeds, obtaining a GI over 80%. The lowest GI was obtained for the control soil for both species of seeds used.

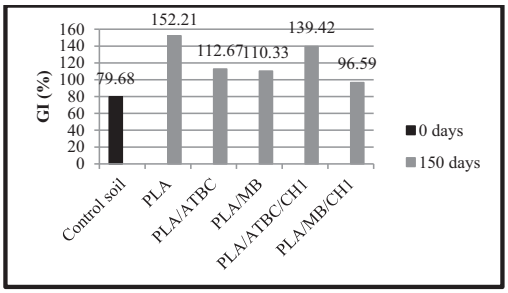

Figure 1. Global germination index (GI) for cucumber seeds

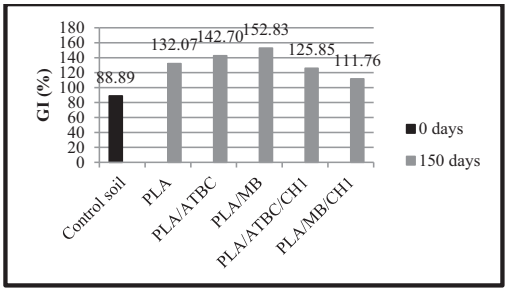

Figure 2. Global germination index (GI) for radish seeds

CONCLUSIONS

The ecotoxic effect of different biodegradable materials based on PLA was studied and the results obtained depend on materials composition, the nature of the biodegradable ingredients and the proportions between them. Depending on their composition, sometimes the materials presented a phytotoxic effect (Souza et al., 2013), and in other situations, depending on the nature of the utilised compounds, the studies showed that the materials did not present an ecotoxic effect (Palsikowski et al., 2018).

In the present study, the tested soils resulted after the polymeric materials biodegradation tests, proved to have, in general, a nontoxic effect over the radish and cucumber seeds, obtaining a GI over 80%. No direct correlation between supernatant concentration and seed germination and root length was found.

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