Chemosphere 289 (2022) 133132

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Phytotoxicity assessment of biodegradable and non-biodegradable plastics using seed germination and early growth tests

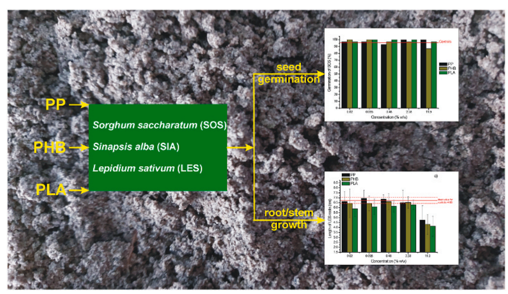
Ewa Liwarska-Bizukojc

Lodz University of Technology, Institute of Environmental Engineering and Building Installations, Al. Politechniki 6, 90-924, Lodz, Poland

HIGHLIGHTS

- Phytotoxicity of bio-based (PLA, PHB) and conventional (PP) plastics was tested.
- Either PLA or PHB or PP did not affect seed germination of higher plants.
- PHB and PLA more often inhibited root growth than PP did.
- Cress was more sensitive and reliable bioindicator than mustard and sorghum.
- Cress is advised to assess the effect of plastics on early stages of plant growth.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Michael Bank

Keywords:
Bio-based plastics
Petroleum-derived plastics
Phytotoxicity
Germination
Growth inhibition
Growth stimulation

ABSTRACT

Global production of plastics remains at the high level despite the SARS-Cov-2 pandemic. These are primarily petroleum-derived plastics but the contribution of bio-based plastics estimated at the level of 1% in the plastic market in 2019 is expected to be increasing. Simultaneously, the significant part of plastic waste is still disposed improperly and pollutes the environment making a threat to all living organisms.

In this work three plastic materials, two bio-based biodegradable: polylactide (PLA) and polyhydroxybutyrate (PHB), and one petroleum-derived non-biodegradable polypropylene (PP) were studied towards their effects on seed germination and early growth of higher plants. The following plants were used as bioindicators: monocotyledonous plant - *Sorghum saccharatum* and two dicotyledonous plants: *Sinapsis alba* and *Lepidium sativum*. Plastics did not affect seed germination of higher plants even at the highest concentration tested (11.9% w/w) but their presence in soil acted in various ways on growth of the plants. Either no or inhibitive or stimulation effects on growth of roots or stems were noticed. It depended on the concentration and chemical composition of the plastic tested, and plant species. PHB and PLA more often caused to the inhibition of root growth than PP did. This phenomenon was observed in particular with regard to the dicotyledonous plants.

Moreover, in the tests with the dicotyledonous plants (*S. alba* and *L. sativum*) the dose-response relations were usually determined as statistically relevant. Among these plants cress (*L. sativum*) occurred to be more sensitive and allowed for obtaining the dose-response dependence for both root and stem length, and, what is important, it took place in the case of each of materials tested. Therefore, cress is recommended to be used as a bioindicator in the assessment of the effect of plastics (petroleum-derived and bio-based plastics) on the early stages of growth of higher plants.

E-mail address: ewa.liwarska-bizukojc@p.lodz.pl.

https://doi.org/10.1016/j.chemosphere.2021.133132
Received 12 October 2021; Received in revised form 18 November 2021; Accepted 29 November 2021
Available online 1 December 2021

1. Introduction

Almost 80% of the total mass of plastic waste (6.3 billion tons) generated from 1950 to 2015 ended up in landfills and the environment (plasticseurope.org). Despite the progress in the management of plastic waste due to the development of the circular economy and promotion of the idea "zero plastic waste to landfill", a significant amount of plastic waste remained still not managed or was directed to the landfills. In 2018 about 30% of generated plastic waste in the world was not collected, i.e. they were disposed under improper conditions or leaked into environment, while about 43% of plastic waste collected globally was managed to landfills (Conversio Market and Strategy, 2020).

In the environment plastic waste are subjected to various physical, chemical and biological processes and, as a result, they are fragmented into macro-, micro- and nanoparticles. Plastic particles of different size and shape (e.g. beads, fragments, fiber, film) are ubiquitous in the aquatic and soil compartments and they are regarded as one of the most common contaminants. About 32% of all plastics produced was estimated to remain in the continental systems (Nizzetto et al., 2016; de Souza Machado et al., 2018). Microplastics were detected in soil in many terrestrial ecosystems (Hu et al., 2021; Piehl et al., 2018; Scheurer and Bigalke, 2018) achieving even the concentration 67.5 g kg^{-1} in the industrial areas in Australia (Fuller and Gautam, 2016). Plastic particles change the physiochemical properties of soil and make a threat to the soil organisms (Rillig et al., 2017, 2019; de Souza Machado et al., 2019; Lozano et al., 2021).

Plants are primary producers that play a key role in the regulation of such ecosystem functions as soil stability and fertility, water availability, composition and distribution of soil microbial community (Chapin, 2003). Due to the direct contact with soil, some species of higher plants are commonly used in the assessment of soil toxicity. With regard to plastics, the ecotoxicological studies concerning *inter alia* the effect of plastic particles on seed germination, biomass growth (e.g. total biomass, root biomass, shoot biomass), root elongation have been performed so far (Qi et al., 2018; de Souza Machado et al., 2019; Balestri et al., 2019; Lozano and Rillig, 2020; Lozano et al., 2021; Huerta Lwanga et al., 2021). In these works the conventional petroleum-derived plastics as well as bio-based plastics were tested. Regarding petroleum-derived plastics polyethylene (PE) and its two types, high-density polyethylene (HDPE) and low-density polyethylene (LDPE), were the most frequently studied to describe the effect of plastics on plants (Balestri et al., 2019; Qi et al., 2018; de Souza Machado et al., 2019; Judy et al., 2019; Lozano et al., 2021). In relation to the bio-based plastics these were starch-based polymers (Balestri et al., 2019; Qi et al., 2018), poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) (Arcos-Hernandez et al., 2012) and polylactide (PLA) (Huerta Lwanga et al., 2021). Garden cress (*Lepidium sativum*) and wheat (*Triticum aestivum*) belong to the most often used higher plants in the ecotoxicological studies for the evaluation of the potential impacts of plastics on primary producers (Qi et al., 2018; Huerta Lwanga et al., 2021; Balestri et al., 2019; Judy et al., 2019; Bosker et al., 2019).

It was reported that neither petroleum-derived nor bio-based plastics affected seed germination processes in soil (Arcos-Hernandez et al., 2012; Balestri et al., 2019; Judy et al., 2012). However, Balestri et al. (2019) noticed that up to 40% of the seeds germinated in the presence of plastic (HDPE or compostable Mater-bi®) leachates showed the developmental abnormalities indicating on leachate phytotoxic effects. With regard to the effect of plastics on the growth of plants, the results were not as clear as it was found in the case of seed germination. de Souza Machado et al. (2019) reported that microplastics might decrease or increase the total dry biomass of onion bulb dependent on the type of microplastics. However, none of six tested microplastics: polyamide (PA), polyester (PES), polyethylene high density (PEHD), polypropylene (PP), polystyrene (PS), poly (ethylene terephthalate) (PET) significantly decreased total biomass of onion bulbs (de Souza Machado et al., 2019). Lozano et al. (2021) revealed that the presence of microplastics (PES, PA, PP, LDPE, PET, polyurethane (PU), PS, polycarbonate (PC)) in soil irrespective of their shape contributed to the increase of root and shoot biomass. Also Huerta Lwanga et al. (2021) did not observe any significant effects of PLA mixed with composts on growth of *Triticum aestivum*. Qi et al. (2018) found that macro- and micro-plastic residues of LDPE and starch-based biodegradable mulch films influenced negatively the growth of above-ground and below-ground parts of wheat and affected both vegetative and reproductive growth. Starch-based biodegradable plastic mulch film showed more severe effects on wheat growth than LDPE film with regard to macroparticles and microparticles (Qi et al., 2018). Microplastics showed stronger negative effects on wheat growth than macroplastics did (Qi et al., 2018). Balestri et al. (2019), who also compared the effect of petroleum-derived plastic (HDPE) and bio-based compostable plastic (Mater-bi®), observed that the hypocotyl was the most sensible seedling organ to HDPE bag leachates, whereas the radicle was the most sensitive to the compostable ones.

Global plastic production increased in the recent decades and even the SARS-Cov-2 pandemic did not cause to the significant drop in this industry sector. It was found that the plastic production decreased only by 0.3% in 2020 (https://www.statista.com/statistics/282732/global-production-of-plastics-since-1950/). Currently produced plastics include fossil fuel-based as well as bio-based plastic materials. They can be biodegradable or not susceptible for microbial decomposition processes. Although bio-based plastics made up about 1% of the global plastic market, the continuous increase of their contribution is expected. It induces the question about the impact of bio-based polymers (biodegradable and non-biodegradable) on the ecosystems, in particular on their biotic part. Plants as producers are the foundation of the biotic part of terrestrial ecosystems. It is hypothesized that bio-based and petroleum-derived plastic particles would not affect the seed germination of plants, however they would influence the early life plant stages. The degree to which the plastic materials would affect the plant growth would depend on the composition of the material and on the plant species used as bioindicator. In order to verify these hypotheses the effect of bio-based (polylactic acid and polyhydroxybutyrate) and petroleum-derived (polypropylene) plastics of different chemical composition and different susceptibility for biodegradation on seed germination and early growth of higher plants was examined.

2. Materials and methods

2.1. Plastic materials

Three plastic materials, two bio-based biodegradable and one petroleum-derived non-biodegradable, available commercially, were purchased from Sigma-Aldrich Ltd. (Germany). These were the following pure polymeric compounds: polylactic acid (PLA), polyhydroxybutyrate (PHB) and polypropylene (PP). All tested materials were in the form of granules of the dimensions from 3 to 5 mm. It means that they can be classified as microplastics. In the case of each of three compounds tested the ecological information is not included in the section 12 of Materials Safety Data Sheet (MSDS).

2.2. Phytotoxicity test

In order to evaluate the potential environmental risk resulting from soil pollution by plastic waste the phytotoxicity test based on germination and seedling growth of the vascular plants was carried out. It was the commercial toxicity bioassay – Phytotoxkit Solid Samples provided by Microbiotests (Belgium) that is conformed with ISO Standards 18,763 (ISO 18763, 2016). The phytotoxkit test allows for the determination of the number of germinated seeds and the growth of roots and shoots after 72 h of the exposure of seeds of selected higher plants to the contaminated matrix in comparison to the controls in a reference soil. In this work the reference OECD soil consisting of air-dried quartz sand (85%), kaolin clay (10%), sphagnum peat (5%) and calcium carbonate ($CaCO_3$)

required to obtain the initial pH level of 6.0 ± 0.5 was used (Microbiotests, Belgium). Plastic particles were added to the reference OECD soil to receive the final concentration: 0.02, 0.095, 0.48, 2.38 and 11.9% w/w. Each concentration was tested in three replications for each plastic material and each plant, while the control tests were made in nine replications for each plant. In this work the following higher plants were used: the monocotyledonous plant *Sorghum saccharatum* (sorghum, series no. SOS041019) and the dicotyledonous plants *Lepidium sativum* (garden cress, series no. LES260820) and *Sinapis alba* (mustard, series no. SIA020719). The plant species were selected because of their rapid germination and growth of their roots and stems. All seeds of these plants were calibrated and provided by Microbiotests (Belgium). Ten seeds of the same test plant were located on the top of wet black paper filter in one row and at the equal distance from each other. Before that the black paper filters were put on the top of the hydrated (control and test) soils in all test plates and waited to become completely wet. After closing, the test plates were put vertically in the holders and located in the acclimation chamber FITO 700 (Biogenet, Poland). They were incubated for 72 h at 25 ± 1 °C in the darkness. Then, a digital picture of each plate was made and transferred to a computer. The length of roots and stems was measured with the help of the image analysis software, i.e. NIS ELEMENTS AR software (Nikon, Japan). The number of germinated seeds was also recorded in each test and control plate. It was assumed that the appearance of a root of at least 1 mm of length indicated that the seed had germinated. Germination index (GI) was calculated as the ratio of the number of germinated seeds to the total number of seeds exposed to the test or control soil in the test plate. The ratio was multiplied by 100%.

2.3. Statistical evaluation of results

The basic statistical elaboration comprising the calculation of mean values, standard deviation and goodness of normal distribution was made with the use of MS Excel. One-way analysis of variance (ANOVA) was applied to evaluate, whether the lengths of roots or stems of plants exposed to one of the plastics tested were statistically equal or different than those that were not exposed to plastics (the control runs without plastic materials in soil). As the null hypothesis it was assumed that they were equal. The confidence level of 95% was assumed. ANOVA implemented in MS Excel (Analysis ToolPak) software was used. In order to describe quantitatively the relation between the concentration of plastics in soil and the length of roots or stems, the series of nonlinear regressions were performed with the help of OriginPro 9.0 (OriginLab). The function dose-response belonging to the category growth/sigmoidal of the following equation was used.

$$L = A_0 + \frac{A_1}{1 + 10^{\log(A_2 - c) - A_3}} \tag{1}$$

where: A_0, A_1, A_2 and A_3 are the parameters of the nonlinear dose-response function, while L is the length of roots (cm) and c is the

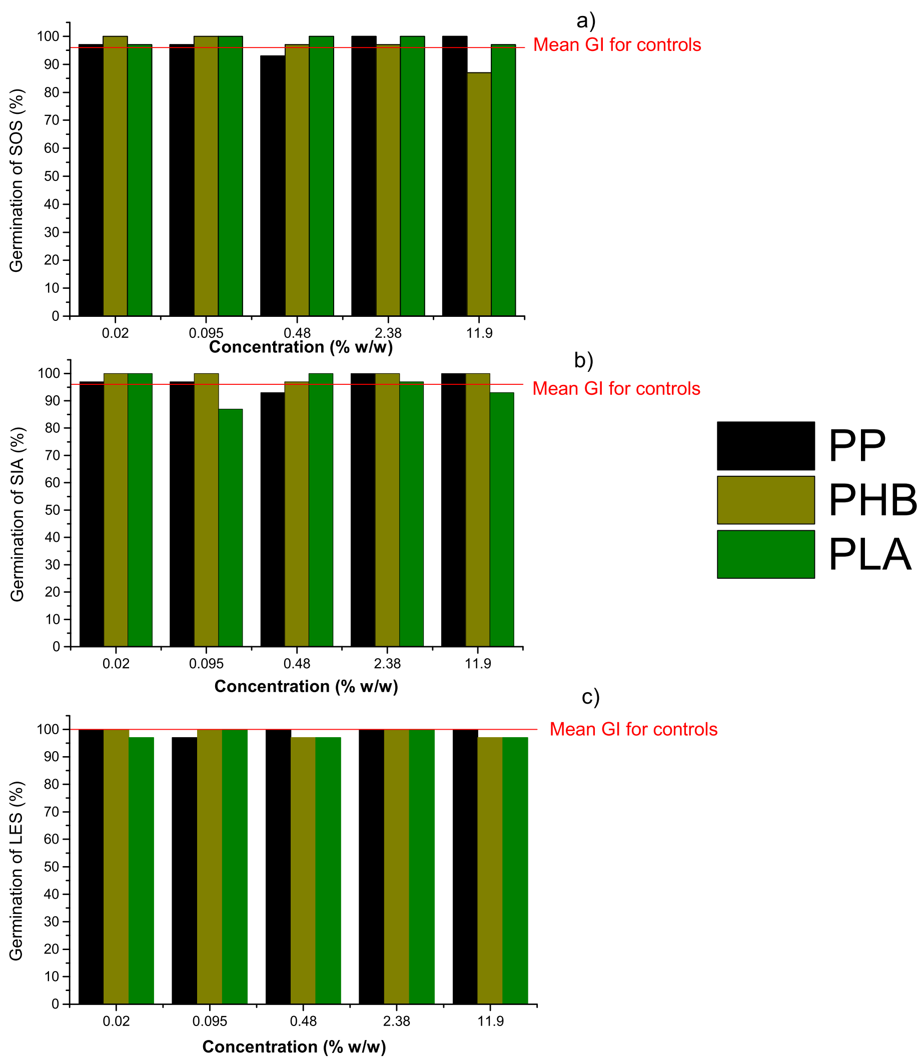


Fig. 1. Effect of plastic materials on the seed germination of the following plants: (a) SOS, (b) SIA and (c) LES.

concentration of plastic particles in soil (% w/w).

3. Results and discussion

3.1. Seed germination

The presence of plastics studied did not influence the germination of any plant used as the bioindicator in the phytotoxicity tests. The values of germination index were from 93 to 100% for SOS and SIA exposed to plastics, while for LES they were from 97% to 100% (Fig. 1). In the control tests the following values of GI were obtained: SOS – 96%; SIA – 96% and LES – 100%. These results showed that there was no difference between the germination of seeds exposed and not exposed to the plastic materials. Similar findings were presented in other works concerning both petroleum-derived as well as bio-based plastics (Arcos-Hernandez et al., 2012; Balestri et al., 2019; Judy et al., 2012). It happens most probably due to the fact that the processes of seed germination are relatively independent of the soil composition because during germination the internal storage materials are used (Milberg and Lamont, 1997; Balestri et al., 2019).

3.2. Effect of plastics on root growth

In Fig. 2 the changes of root length of higher plants exposed to the plastic compounds tested are depicted. The mean values of length of SOS roots in the runs with the studied plastics varied from 3.04 to 5.25 cm, while in the control test the mean length of roots for sorghum was 4.70 cm ± 0.65 cm. For LES and SIA the mean length of roots were in the range from 4.13 to 6.96 cm and from 4.57 to 8.57 cm, respectively. The mean length of roots obtained in the control tests for these plants were as follows: LES – 6.73 cm ± 0.3 cm, SIA – 7.66 cm ± 0.77 cm.

Analyzing the results presented in Fig. 2 it was found that the presence of plastics in soil did not affect or weakly affected the early growth of roots excluding the highest concentration of tested materials in soil (11.9% w/w). At this concentration the inhibitive effect of plastics on root growth was visible primarily with regard to the dicotyledonous plants (Fig. 2). In the case of the monocotyledonous plant the inhibition of growth of sorghum roots was observed in the runs with PHB only (Fig. 2). PP did not inhibit growth of sorghum at any concentration tested (Fig. 2). With regard to PLA it was difficult to judge, whether the inhibition of growth of sorghum roots appeared. It seemed that at higher concentrations, above 0.48% w/w, the root growth of SOS was inhibited (Fig. 2).

In order to confirm the aforementioned observations and systemize them, the results of phytotoxicity tests were subjected to one-way ANOVA (Table 1a–c). For the dicotyledonous plants (LES and SIA) *p*-values were below the assumed significance level ($p = 0.05$) at the highest concentration of plastics in soil and it was determined for all three plastic materials studied (Table 1b–c). The same was found for SOS exposed to PHB at its highest concentration in soil. No statistically relevant differences were determined between the length of SOS roots that were exposed to PLA or PP in relation to the control test (Table 1a). These findings confirmed the inhibition of root growth of dicotyledonous plants at the highest concentration (11.9% w/w) of each plastic tested and the inhibition of sorghum roots exposed to PHB at the concentration of 11.9% w/w. It should be added that the highest concentration studied in this work exceeded the concentrations of plastics in soil environment reported in the literature that did not exceed 6.75% w/w (Fuller and Gautam, 2016). However, it allowed for the amplification of the effect of plastics on producers in the terrestrial ecosystems. Such approach is often made in the ecotoxicological tests.

Comparing the impact of plastics studied on root growth, it was noticed that bio-based plastics (PHB and PLA) acted stronger than the petroleum-based plastic (PP) did. In particular it was well seen in the tests with PLA towards cress (Fig. 2c and Table 1c). The inhibition of growth of LES roots exposed to PLA was confirmed for almost each

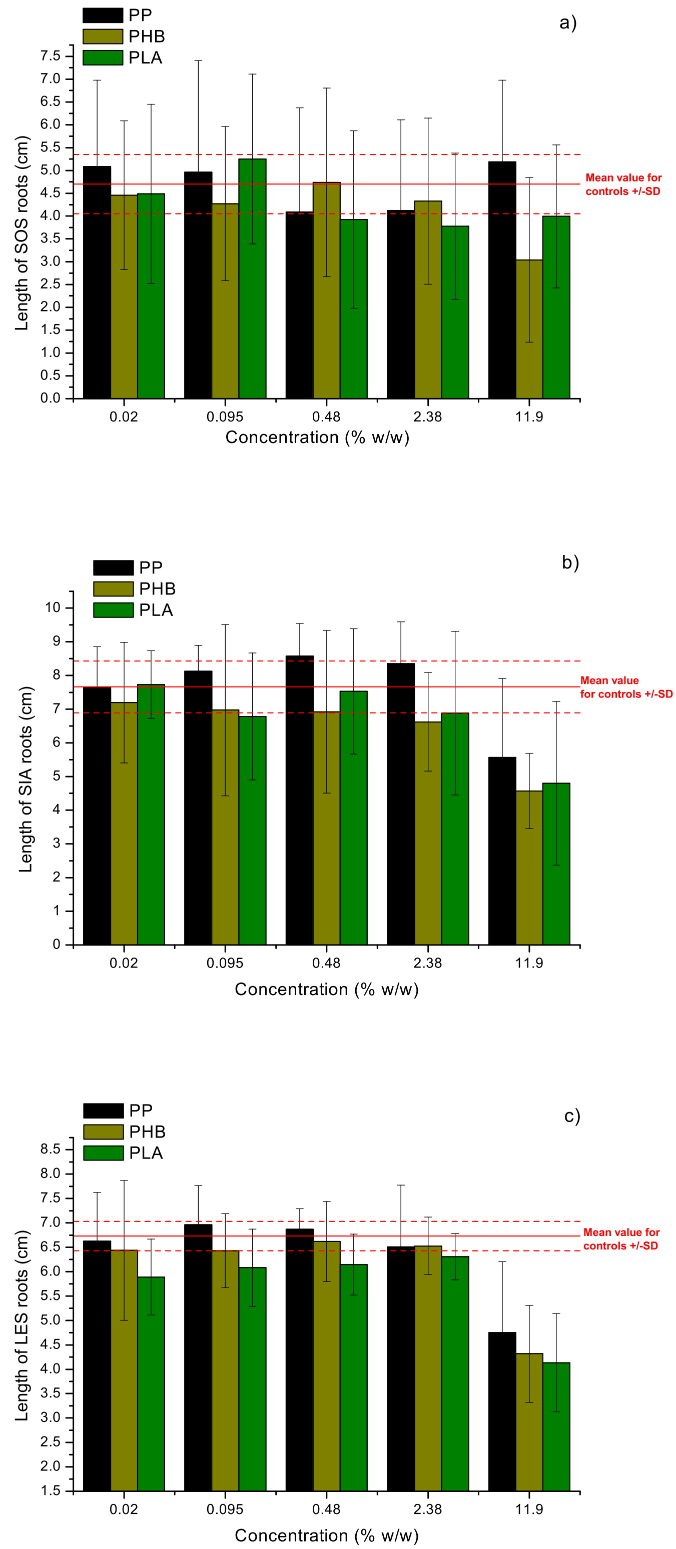


Fig. 2. Effect of plastic materials on the root elongation of the following plants: (a) SOS, (b) SIA and (c) LES.

studied concentration of PLA in soil (Table 1c). It is in line with the observations made by Balestri et al. (2019), who found that the radicles were more sensitive to the presence of compostable plastic than the conventional one (HDPE). Petroleum-based plastics usually do not influence root growth (de Souza Machado et al., 2019), however it depends on their concentration in soil (Lozano et al., 2021). de Souza

Table 1a
Results of one-way ANOVA for SOS.

Tested compound	Plant organ	*p*-values for SOS				
		Concentrations (% w/w)				
		0.02	0.095	0.48	2.38	11.9
PP	roots	0.4112	0.5923	0.1648	0.1739	0.2804
	stems	0.2712	0.7686	0.2499	0.06252	0.05249
PHB	roots	0.5367	0.2979	0.9639	0.3698	0.000155 (I)
	stems	0.3451	0.03224 (I)	0.9481	0.5557	0.001095 (I)
PLA	roots	0.5940	0.2218	0.07030	0.02804 (I)	0.07958
	stems	0.5464	0.9352	0.01265 (I)	0.04140 (I)	0.04097 (I)

(I) - inhibition; (S) - stimulation.

Table 1b
Results of one-way ANOVA for SIA.

Tested compound	Plant organ	*p*-values for SIA				
		Concentrations (% w/w)				
		0.02	0.095	0.48	2.38	11.9
PP	roots	0.935	0.213	0.0177(S)	0.0659	$7.79 \cdot 10^{-7}$ (I)
	stems	0.821	0.0505	0.941	0.974	0.000201 (I)
PHB	roots	0.207	0.0921	0.0669	0.00497 (I)	$1.52 \cdot 10^{-14}$(I)
	stems	0.0543	0.0532	0.869	0.0460 (I)	0.00192 (I)
PLA	roots	0.889	0.0312(I)	0.697	0.0551	$1.73 \cdot 10^{-10}$ (I)
	stems	0.0283 (S)	0.0744	0.0586	0.400	0.00443 (I)

(I) - inhibition; (S) - stimulation.

Table 1c
Results of one-way ANOVA for LES.

Tested compound	Plant organ	*p*-values for LES				
		Concentrations (% w/w)				
		0.02	0.095	0.48	2.38	11.9
PP	roots	0.573	0.207	0.418	0.262	$1.19 \cdot 10^{-16}$ (I)
	stems	0.0434 (S)	0.000104 (S)	0.209	0.0283 (I)	$8.47 \cdot 10^{-12}$ (I)
PHB	roots	0.156	0.0917	0.581	0.248	$3.82 \cdot 10^{-17}$ (I)
	stems	0.0837	0.0563	0.378	$6.38 \cdot 10^{-15}$ (I)	$5.99 \cdot 10^{-15}$ (I)
PLA	roots	$8.71 \cdot 10^{-6}$ (I)	0.000397 (I)	0.00119 (I)	0.118	$3.69 \cdot 10^{-15}$ (I)
	stems	0.0447 (S)	$2.53 \cdot 10^{-5}$ (S)	0.000295 (S)	0.927	$7.37 \cdot 10^{-6}$ (I)

(I) - inhibition; (S) - stimulation.

Machado et al. (2019) reported that PP at concentration of 2% w/w in soil exerted a weak effect on the root biomass of *Allium fistulosumand.* At the same time no significant differences between the values of root biomass obtained in the tests with microparticles of PP and the control tests were found (de Souza Machado et al., 2019).

In order to describe properly the dependence between the concentrations of plastics and the length of roots, the dose-response relations were sought for each plant and each material. The values of correlation coefficients (R^2) are presented in Table 2. It was assumed that the critical value of correlation coefficient at $p = 0.05$ with four degrees of freedom is 0.811 and it must be exceeded to consider the correlation significant. Taking it into account, the correlations determined for LES exposed to each material studied and for SIA exposed to PHB were regarded statistically relevant (Table 2). It showed that the cress was most probably the best bioindicator used in the phytotoxicity tests aiming at the evaluation of the effect of plastics on early growth of roots. In Fig. 3 the dose-dependence correlations for the roots of cress were depicted as an example.

Table 2
The values of correlation coefficient R^2 for dose-response approximation.

Plant		Tested material		
		PP	PHB	PLA
SOS	roots	0.119	0.743	0.124
	stems	0.192	0.183	0.133
LES	roots	0.927	0.975	0.889
	stems	0.904	0.999	0.961
SIA	roots	0.681	0.976	0.631
	stems	0.763	0.962	0.993

3.3. Effect of plastics on stem growth

The presence of PHB, PLA or PP in soil influenced stem growth of higher plants in various ways. It was found that tested plastics (PHB, PLA or PP) caused to either the inhibition or the stimulation of stem growth of plants or they did not affect the growth of stems (Fig. 4).

At the highest concentration each of three plastics contributed to the decrease of length of stems excluding the stems of SOS exposed to PP (Fig. 4 and Table 1a–c). It was similar to the finding for the growth of roots. Inhibition of growth of stems or roots exposed to PHB or PLA might have been caused by the changes of physical properties of soil induced by the presence of these polymers or by the presence of products of microbial decomposition of PHB and PLA in soil or by these two reasons simultaneously. Taking the duration of the phytotoxicity tests (72 h) into account, the biodegradation of biopolymers could only start. It was determined that microbial decomposition of PLA or PHB took usually two or three weeks under favorable conditions (Altaee et al.,

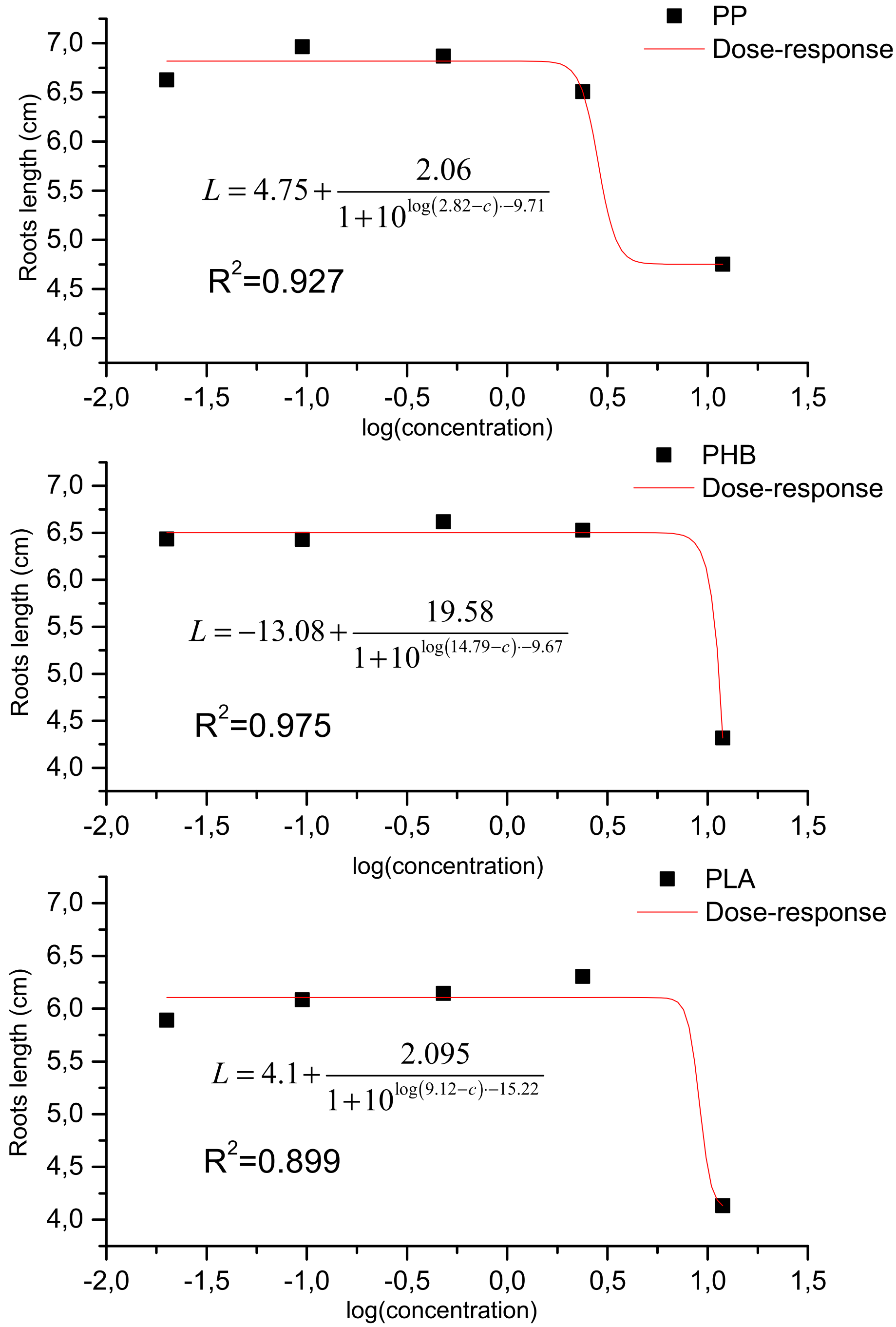


Fig. 3. An example of the determination of dose-response correlations for the roots of *Lepidium sativum*.

2016; Pattanasuttichonlakul et al., 2018; Zaaba and Jaafar, 2020; Martínez-Tobón et al., 2018).

The stimulation effect on stem length was observed in the tests with two out of three tested plastics, i.e. PP and PLA. In the case of PP, the stimulation of stem growth appeared regarding SOS and LES at various concentrations of PP in the soil. It is difficult to interpret this phenomenon because PP is generally considered as a polymer of good chemical and cracking resistance (Tripathi, 2002). It is hardly possible that any degradation products of PP might have been released within three days. Thus, the phenomena like the inhibition or stimulation of growth of plant organs exposed to PP resulted most probably from the changes of physical properties of soil containing PP. It was proved that the presence of plastics in soil influenced *inter alia* soil density, pore space, soil moisture, evaporation, water holding capacity (de Souza Machado et al., 2018, 2019; Guo et al., 2020; Wan et al., 2019). These changes in soil properties may lead to the positive effects as, for example, better aeration of soil (Rillig et al., 2019; Lozano et al., 2021) as well as to the negative effects like water loss and soil drying (de Souza Machado et al., 2019; Wan et al., 2019). PLA stimulated stem growth of dicotyledonous plants only, in particular it concerned the stems of cress at the concentration up to 0.48% w/w. With regard to the monocotyledonous plant, the inhibition of stem growth exposed to PLA at the concentrations 0.48% w/w and higher was observed (Fig. 4 and Table 1a). PHB either did not act on stem growth of higher plants or inhibited it (Fig. 4). This polymer contributed to the decrease of stem length of dicotyledonous plants (SIA and LES) at two highest concentrations of PHB in soil (Table 1b–c). It proved again that dicotyledonous plants at early growth stages occurred to be more sensitive organisms than monocotyledonous

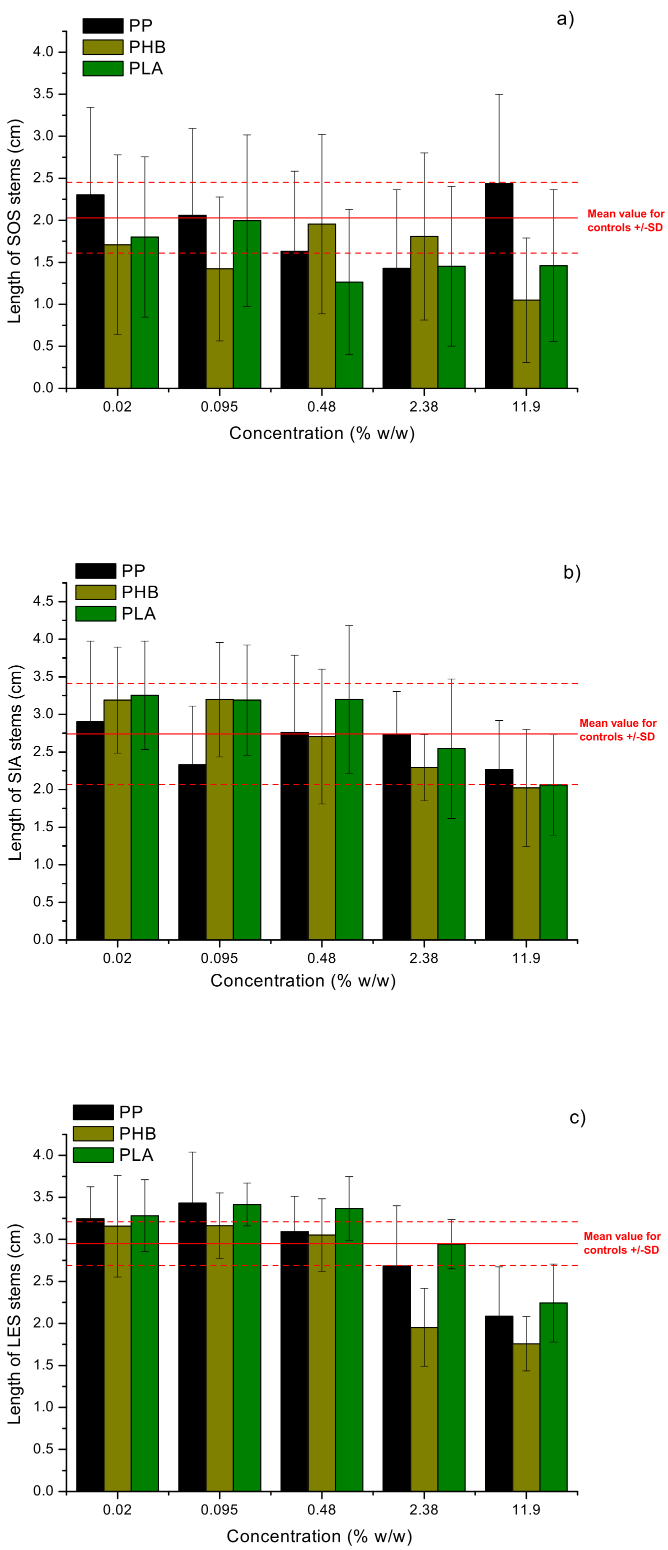


Fig. 4. Effect of plastic materials on the stem elongation of the following plants: a) SOS, b) SIA and c) LES.

plants.

Analyzing the correlation between the concentration of plastics in soil and the length of stems, it was found that dose-response dependencies were statistically significant only for the dicotyledonous plants, i.e. cress and mustard (Table 2). Similarly as it was stated for the length of roots of LES, the dose-response relation was also determined for stems of LES regarding each plastic material tested (Table 2). It makes LES the most sensitive and the most reliable plant for testing the effect of plastics on higher plants. With regard to stems also SIA occurred to be a good bioindicator because for two materials, PHB and PLA, the dose-response relation was confirmed as statistically relevant (Table 2).

4. Conclusions

Plastics (PHB, PLA and PP) do not affect seed germination of higher plants even at the high concentrations in the soil environment (11.9% w/w).

The presence of plastics (PHB, PLA, PP) in soil acts in various ways on growth of plant organs. Plastics may cause to the inhibition or stimulation of root or stem growth as well as they may not affect the growth of plants. It depends primarily on the concentration of plastic compound, and then it depends on the chemical composition of plastics and plant species.

Dicotyledonous plants are more sensitive to the exposure of plastics than monocotyledonous plants. Subsequently, they are better bioindicators for the assessment of the effect of plastics on the early growth of higher plants.

Although the results of phytotoxicity tests characterize the variability, the dose-response dependence is determined as statistically relevant in the tests with dicotyledonous plants (*S. alba* and *L. sativum*). In the case of *L. sativum* the dose-response dependence is found for both root and stem length, and, what is important, for each of materials tested.

Lepidium sativum is recommended to be used as a bioindicator in the assessment of the phytotoxic effects of plastics on the early growth of higher plants.

Author contribution

Ewa Liwarska-Bizukojc - conceptualization, data curation, investigation, visualisation, writing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was supported by the European Union's Horizon 2020 - Research and Innovation Framework Programme through the research project BIO-PLASTICS EUROPE (Grant agreement No. 860407) and Institute of Environmental Engineering and Building Installations of Lodz University of Technology (Poland).

References

Altaee, N., El-Hiti, G.A., Fahdil, A., Sudesh, K., Yousif, E., 2016. Biodegradation of different formulations of polyhydroxybutyrate films in soil. SpringerPlus 5, 762. https://doi.org/10.1186/s40064-016-2480-2.

Arcos-Hernandez, M.V., Laycock, B., Pratt, S., Donose, B.C., Nikolić, M.A.L., Luckman, P., Werker, A., Lant, P.A., 2012. Biodegradation in a soil environment of activated sludge derived polyhydroxyalkanoate (PHBV). Polym. Degrad. Stabil. 97, 2301–2312. https://doi.org/10.1016/j.polymdegradstab.2012.07.035.

Balestri, E., Menicagli, V., Ligorinia, V., Fulignati, S., Raspolli Galletti, A.M., Lardicci, C., 2019. Phytotoxicity assessment of conventional and biodegradable plastic bags using seed germination test. Ecol. Indicat. 102, 569–580. https://doi.org/10.1016/j.ecolind.2019.03.005.

Bosker, T., Bouwman, L.J., Brun, N.R., Behrens, P., Vijver, M.G., 2019. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*. Chemosphere 226, 774–781. https://doi.org/10.1016/j.chemosphere.2019.03.163.

Chapin, F.S., 2003. Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. Ann. Bot. 91, 455–463.

Conversio Market, Strategy GmbH, 2020. Global plastics flow 2018. https://www.carboliq.com/pdf/19_conversio_global_plastics_flow_2018_summary.pdf. (Accessed 12 October 2021).

de Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M. C., 2018. Impacts of microplastics on the soil biophysical environment. Environ. Sci. Technol. 52 (17), 9656–9665. https://doi.org/10.1021/acs.est.8b02212.

de Souza Machado, A.A., Lau, ChW., Kloas, W., Bergmann, J., Bachelier, J.B., Faltin, E., Becker, R., Gorlich, A.S., Rillig, M.C., 2019. Microplastics can change soil properties and affect plant performance. Environ. Sci. Technol. 53, 6044–6052. https://doi.org/10.1021/acs.est.9b01339.

Fuller, S.G., Gautam, A., 2016. A procedure for measuring microplastics using pressurized fluid extraction. Environ. Sci. Technol. 50, 5774–5780. https://doi.org/10.1021/acs.est.6b00816.

Guo, J.J., Huang, X.P., Xiang, L., Wang, Y.Z., Li, Y.W., Li, H., Cai, Q.Y., Mo, C.H., Wong, M.H., 2020. Source, migration and toxicology of microplastics in soil. Environ. Int. 137, 105263. https://doi.org/10.1016/j.envint.2019.105263.

Hu, C., Lu, B., Guo, W.S., Tang, X.Y., Wang, X.F., Xue, Y.H., Wang, L., He, X., 2021. Distribution of microplastics in mulched soil in Xinjiang, China. Int. J. Agric. Biol. Eng. 14 (2), 196–204. https://doi.org/10.25165/j.ijabe.20211402.6165.

Huerta-Lwanga, E., Mendoza-Vega, J., Ribeiro, O., Gertsen, H., Peters, P., Geissen, V., 2021. Is the Polylactic Acid fiber in green compost a risk for *Lumbricus terrestris* and *Triticum aestivum*? Polymers 13, 703. https://doi.org/10.3390/polym13050703.

Soil Quality - Determination of the Toxic Effects of Pollutants on Germination and Early Growth of Higher Plants. Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, 2016.

Judy, J.D., Williams, M., Gregg, A., Oliver, D., Kumar, A., Kookana, R., Kirby, J.K., 2019. Microplastics in municipal mixed-waste organic outputs induce minimal short to long-term toxicity in key terrestrial biota. Environ. Poll. 252, 522–531. https://doi.org/10.1016/j.envpol.2019.05.027.

Lozano, Y.M., Rillig, M.C., 2020. Effects of microplastic fibers and drought on plant communities. Environ. Sci. Technol. 54, 6166–6173. https://doi.org/10.1021/acs.est.0c01051.

Lozano, Y.M., Lehnert, T., Linck, L.T., Lehmann, A., Rillig, M.C., 2021. Microplastic shape, polymer type, and concentration affect soil properties and plant biomass. Front. Plant Sci. 12, 616645. https://doi.org/10.3389/fpls.2021.616645.

Martínez-Tobón, D.I., Gul, M., Elias, A.L., Sauvageau, D., 2018. Polyhydroxybutyrate (PHB) biodegradation using bacterial strains with demonstrated and predicted PHB depolymerase activity. Appl. Microbiol. Biotechnol. 102 (18), 8049–8067. https://doi.org/10.1007/s00253-018-9153-8.

Milberg, P., Lamont, B.B., 1997. Seed/cotyledon size and nutrient content play a major role in early performance of species on nutrient-poor soils. New Phytol. 137, 665–672. https://doi.org/10.1046/j.1469-8137.1997.00870.x.

Nizzetto, L., Futter, M., Langaas, S., 2016. Are agricultural soils dumps for microplastics of urban origin? Environ. Sci. Technol. 50, 10777–10779. https://doi.org/10.1021/acs.est.6b04140.

Pattanasuttichonlakul, W., Sombatsompop, N., Prapagdee, B., 2018. Accelerating biodegradation of PLA using microbial consortium from dairy wastewater sludge combined with PLA-degrading bacterium. Int. Biodeterior. Biodegrad. 132, 74–83. https://doi.org/10.1016/j.ibiod.2018.05.014.

Piehl, S., Leibner, A., Löder, M.G.J., Dris, R., Bogner, C., Laforsch, C., 2018. Identification and quantification of macro- and microplastics on an agricultural farmland. Sci. Rep. Dec. 18 8 (1), 17950. https://doi.org/10.1038/s41598-018-36172-y.

Qi, Y., Yang, X., Pelaez, A.M., Huerta Lwanga, E., Beriot, N., Gertsen, H., Garbeva, P., Geissen, V., 2018. Macro- and micro- plastics in soil-plant system: effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. Sci. Total Environ. 645, 1048–1056. https://doi.org/10.1016/j.scitotenv.2018.07.229.

Rillig, M.C., Ingraffia, R., de Souza Machado, A.A., 2017. Microplastic incorporation into soil in agroecosystems. Front. Plant Sci. 8, 1805. https://doi.org/10.3389/fpls.2017.01805.

Rillig, M.C., Lehmann, A., de Souza Machado, A.A., Yang, G., 2019. Microplastic effects on plants. New Phytol. 223 (3), 1066–1070. https://doi.org/10.1111/nph.15794.

Scheurer, M., Bigalke, M., 2018. Microplastics in Swiss floodplain soils. Environ. Sci. Technol. 52, 3591–3598. https://doi.org/10.1021/acs.est.7b06003.

Tripathi, D., 2002. Practical Guide to Polypropylene. Rapra Technology Ltd, Shawbury, U.K.. ISBN 978-1859573457.

Wan, Y., Wu, Ch, Xue, Q., Hui, X., 2019. Effects of plastic contamination on water evaporation and desiccation cracking in soil. Sci. Total Environ. 654, 576–582. https://doi.org/10.1016/j.scitotenv.2018.11.123.

Zaaba, N.F., Jaafar, M., 2020. A review on degradation mechanisms of polylactic acid: hydrolytic, photodegradative, microbial, and enzymatic degradation. Polym. Eng. Sci. 60/9, 2061–2075. https://doi.org/10.1002/pen.25511.