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Study on microbioplastic leachates: Released compounds and impact on higher plant growth

Ewa Liwarska-Bizukojć[1,*], Marcin Bizukojć[2]

[1]Institute of Environmental Engineering and Building Installations, Lodz University of Technology, Al. Politechniki 6, 90-924 Łódź, Poland
[2]Department of Bioprocess Engineering, Faculty of Process and Environmental Engineering, Lodz University of Technology, Wólczańska 213, 93-005 Łódź, Poland

Corresponding author, e-mail: ewa.liwarska-bizukojc@p.lodz.pl
ORCID numbers
Ewa Liwarska-Bizukojć 0000-0003-4510-1369
Marcin Bizukojć 0000-0003-1641-9917

Abstract. The effects of leachates from newly-synthesized bioplastics on the early stages of higher plant growth were studied together with the putative identification of the chemicals in the given microbioplastic leachates. Three polylactide-based bioplastics and pure polylactide (PLA) were subjected to the phytotoxicity tests (1) to determine the intrinsic effects of chemicals on the germination and early growth of plants without prior incorporation of the chemicals into a soil and (2) to find the impact of the chemicals introduced into a soil on the germination and plant growth. Plants *Sorghum saccharatum*, *Lepidium sativum* and *Sinapis alba* were used. For two out of four microbioplastics the total ion chromatograms revealed the presence of chemicals in the leachates. Out of 20 individual m/z values, 6 were putatively attributed to the known compounds. Microbioplastic leachates did not affect seed germination and contributed rather to the stimulation than inhibition of the early plant growth. In the soil

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tests the inhibition of root and shoot growth of dicotyledons occurred more frequently than in the liquid phase tests. It indicates the potential interactions between the chemicals in the leachates and soil matrix. Dicotyledons were more sensitive than monocotyledons in the evaluation of phytotoxicity of microbioplastic leachates.

Keywords: bioplastic; inhibition; leachate; mass spectrometry; plants

1. INTRODUCTION

Bioplastics, similarly as petroleum derived plastics, are a specific group of carbon based (organic) polymers. Apart from monomeric ingredients they usually contain additives that modify the properties of pure polymers to improve the physicochemical characteristics of the final product, e.g. increase pliability and reduce flammability. Additives can be classified on the basis of their functional and structural components into four groups: functional additives, colorants, fillers and reinforcements (Hansen et al., 2013; Gunaalan et al., 2020). To functional additives belong primarily plasticizers without whom plastics would not be as useful as they are. Colorants include pigments that are used to dye the products. Fillers include such substances as clay, talc or carbonates added to improve polymer coating properties, while stabilizers include synthetic fibers used to increase product mechanical resistance (Hansen et al., 2013; Gunaalan et al., 2020). The European Chemical Association characterised over 400 substances used as plastic additives (ECHA, 2018; Gunaalan et al., 2020). Plasticizers are widely used compounds that made another compound, usually a plastic, more pliable (EPA, 2016). The majority of plasticizers are esters, specifically, phthalates and adipates. Some of the phthalates that were often used in plastics in the past include dibutylphthalate (DBP), diethylhexylphthalate (DEHP), dimethylphthalate (DMP), and benzylbutylphthalate (BBP) (Oehlmann et al., 2009; EPA, 2016). Bisphenol A (BPA) was also a commonly added plasticizer (EPA, 2016). Apart from known chemical additives also a number of non-intentionally added substances (NIAS) of unidentified composition might be included in plastics. The additives most frequently make a few percentages of the polymer weight, e.g. biocides or antistatics are usually at the levels of 1-2%; while colorants range from 1 to 4%, The other additives are used at much higher contents: thermal stabilizers up to 8%; flame retardants from 10 to 20%; plasticisers from 10 to 70%; fillers up to 50% (Andrady and Rajapakse, 2019; Gunaalan et al., 2020).

Plastic as well as bioplastic waste abandoned in the environment is exposed to changing weather conditions (e.g. rainfall, UV radiation, temperature), mechanical, chemical and biological processes (Guo et al., 2020). As a result it is progressively degraded and fragmented into micro- and nanoplastic particles. It promotes leaching and release of additives into the environment. They are easily released because they are not covalently bound to the polymers (Zimmermann et al., 2021; Gao et al., 2022, Gunaalan et al., 2022). Leachates are a mixture of chemicals and their composition may change dependent on weather conditions, time, pH and other physicochemical properties of aquatic and/or soil environment. Also the toxicity of plastic leachate depends on its composition and may vary widely as even different additives of different amount to the same polymer might be added to achieve the sufficient functionality of the final product (Gao et al., 2022).

Studies concerning the release of additives from plastic materials have been published since the 1990s (*inter alia* Berens, 1997; Gunaalan et al., 2022). However, the evaluation of the ecological risk caused by plastic leachates has been started about a decade later. Lithner et al. (2009) claimed that leaching tests on plastics with subsequent toxicity tests had never been reported before they published their work. So far the effect of plastic leachate on living organisms have been tested primarily with regard to aquatic biota, in particular marine organisms (Lithner et al., 2012; Gao et al., 2022, Gunaalan et al., 2022). For example, Capolupo et al. (2020) observed that all types of leachates produced from car tire rubber (CTR), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC) inhibited the growth of marine and freshwater microalgae, i.e. *Skeletonema costatum* and *Raphidocelis subcapitata*, respectively. In another work it was found that PVC plastics containing diisononylphthalate (DiNP) contributed to the increase in body length and the decrease in offspring of *Daphnia magna* (Schrank et al., 2019). The leachates from the conventional undegradable bags (high density polyethylene, HDPE) and compostable bags affected seed germination and seedling growth of the coastal dune plants *Thinopyrum junceum* and *Glaucium flavum* (Menicagli et al., 2019, Gunaalan et al., 2022).

While the data on the effect of plastic leachates on aquatic biota are limited as indicated inter alia in the critical review by Gunaalan et al. (2022), the ecotoxicological data on the potential impacts of plastic and/or bioplastic leachates on soil organisms have not been published according to our best knowledge so far.

In this work four types of leachates obtained from different microbioplastics, three newly-synthesized polylactide-based bioplastics and one pure polylactide (PLA) were studied towards their impact on the early stages of higher plant growth. Additionally, the products

leached from microbioplastics were putatively identified. The hypothesis that bioplastic leachates would not affect the seed germination of plants, but instead they would stimulate or inhibit the early growth of soil plants was verified here.

2. MATERIALS AND METHODS

2.1. Bioplastics

In all four bioplastics studied PLA was the main component. Three of them referred to as PLA-based bioplastics were obtained as a result of the cooperation within Bio-plastic Europe Project (Horizon 2020, grant agreement No. 860407) and were manufactured for specific applications, i.e. agriculture, cutlery and packaging, while one was pure PLA delivered by Sigma-Aldrich Ltd. (Germany). The latter was classified as a reference material in this study. All bioplastics studied were in the form of microparticles, i.e. granules of dimensions up to 4 millimetres. Regarding the nomenclature assumed in the project three following PLA-based bioplastic materials were examined: BPE-AMF-PLA (Bio-Plastic Europe - Agriculture Mulch Film - PolyLactic Acid) provided by NaturePlast SAS (NP, France), BPE-C-PLA (Bio-Plastic Europe-Cutlery-PolyLactic Acid and BPE-RP-PLA (Bio-Plastic Europe - Rigid Packaging - PolyLactic Acid) provided by Arctic Biomaterials OY Ltd. (ABI, Finland). In the case of BPE-AMF-PLA PLA that made 50-70% of this material was blended with polybutylene adipate terephthalate (PBAT) (the content of about 15%). Also the chemical additives (<5%) were added to make BPE-AMF-PLA useful for extrusion applications. BPE-C-PLA contained about 20% of degradable glass fibres apart from PLA (50-80%), while BPE-RP-PLA was composed of PLA (50-80%) and a mineral filling compound (food grade) for injection, molding and potentially sheets for thermoforming. More information about these three PLA-based bioplastics was presented elsewhere (Liwarska-Bizukojc, 2023).

2.2. Batch leaching tests

A methodology of plastic leaching test described by Lithener et al. (2009) was adopted. Deionised water was used as a leaching medium. A 20 g of bioplastic microparticles of one of the bioplastics studied was placed in a glass Erlenmeyer flask of the total volume of 300 ml. Then 200 ml of deionised water was added to obtain the concentration of 100 g bioplastic material per litre, equivalent to a liquid to solid ratio (L/S) of 10 L kg^{-1} (Lithener et al., 2009). Three Erlenmeyer flasks containing only deionised water were prepared in parallel. Three replications were made for each bioplastic material tested as well as for the control sample

(deionised water). All flasks were located in a rotary shaker Certomat® IS and the speed was set to 90 rpm. Shaking was performed at the constant temperature 20±0.5°C in the darkness for 14 days. Then the samples were left to settle and the plastic leachates were separated and subjected to further examinations. As a result of this test four types of plastic leachates, i.e. one for each of bioplastic tested were produced.

2.3. Phytotoxicity tests

In order to evaluate phytotoxic effect of bioplastic leachates on the germination and the early growth of higher plants two types of tests were applied. These tests called Phytotestkit and Phytotoxkit Solid Samples and they were provided by Microbiotests (Ghent, Belgium). Phytotestkit allows for the determination of the "direct" (intrinsic) effects of chemicals on the germination and early growth of plants without prior incorporation of the chemicals into a (reference) soil, whereas in the Phytotoxkit Solid Samples the chemical is first introduced into the soil and then its potential impact on seed germination and plant growth is tested. In both tests three plants, i.e. monocotyledonous plant *Sorghum saccharatum* (sorghum, series no. SOS041019) and two dicotyledonous plants *Lepidium sativum* (garden cress, series no. LES260820) and *Sinapis alba* (mustard, series no. SIA020719) were used as model organisms. They were cultured in the specially designed transparent test plates (21.0×15.5×0.8 cm). The tests for each plastic material and each plant were made in three replications, whereas the control tests with deionised water were made in six replications for each plant. Below the methodologies of both tests are described.

2.3.1. Phytotestkit

In this test 20 ml of deionised water (the control test) or one type of bioplastic leachate was slowly spread over the entire surface of the thick white filter paper that was previously located on the foam pad and parafilm sheet. When the white filter was completely hydrated the thin black filter paper was placed on it. When the black filter became completely wet 10 seeds of one of the three plants used as model organisms were placed on it in one row and at equal distance of each other. Then the cover was carefully placed on the bottom part of the test plate and the test plate was closed. All test plates were vertically positioned in the holders and incubated for 72 h at 25±1°C in the darkness in the acclimation chamber FITO 700 (Biogenet, Józefów, Poland).

2.3.2. Phytotoxkit Solid Samples

The reference OECD soil delivered by Microbiotests (Belgium) was applied. 90 ml of the OECD reference soil was placed in the test plate. Then, the soil was hydrated using either the plastic leachate or deionised water (the control test). After that the black paper filter was placed on the top of the hydrated soil. When it became completely wet, the seeds (10) of one of the three plants used as model organisms were placed on it in one row in the same way as it was made in the Phytotestkit. The test plates were closed and vertically positioned in the holders. Then they were incubated in the acclimation chamber FITO 700 (Biogenet, Józefów, Poland) and incubated for 72 h at 25±1 °C in the darkness. The procedure of Phytotoxkit Solid Samples test complies with ISO Standards 18763 (ISO, 2016).

2.3.3. Measurements and calculations

For each test plate the number of germinated seeds was recorded. Based upon these data the germination percentage for each sample was calculated. Also a digital picture of each test plate was made and the lengths of roots and shoots were measured with the help of image analysis using the NIS ELEMENTS AR software (Nikon, Japan).

The mean values and standard deviations of the germination percentage, the length of roots, and the length of shoots were calculated. In order to check whether the lengths of roots or shoots of plants exposed to one type of the plastic leachates tested were statistically equal or different than those that were not exposed to the plastic leachates one-way analysis of variance (ANOVA) at statistical significance $\alpha = 0.05$ was used. As the null hypothesis it was assumed that they were equal. One-way ANOVA was preceded by checking the assumptions required for the parametric tests including the normality of data, which was verified with the help of Kolmogorov-Smirnov test. The mean values and standard deviations were calculated using MS Excel (Analysis ToolPak) software. MS Excel (Analysis ToolPak) was also used for one-way ANOVA. Kolmogorov-Smirnov test was performed with the help of OriginPro 9.0 (OriginLab) software. OriginPro 9.0 (OriginLab) was employed for the visualisation of the results.

2.4. Analysis of the composition of plastic leachates

The substances released from the studied bioplastics were analysed with the use of liquid chromatography (UPLC® Aquity) coupled with mass spectrometry Synapt 2G (Waters, USA). A Waters Acquity UPLC® BEH Shield RP18 column (2.1 mm×100 mm×1.7 mm) at the gradient elution of acetonitrile and water (both solvents acidified with 0.1% formic acid) was

used. The gradient elution was designed as follows (CH_3CN:H_2O): 0.0–2.5 min 0:100 (v/v), 2.5–3.5 min 20:80 (v/v), 3.5–4.5 min 30:70 (v/v), 4.5–6.8 min 40:60 (v/v), 6.8–14.0 min 60:40 (v/v). The mass spectrometry analysis was conducted both in positive and negative electrospray ionisation modes. The following parameters were applied: the temperature of the source was set to 120°C; desolvation temperature was 200°C in ESI+ mode and 400°C in ESI− mode; voltage was 3 kV for the capillary, 40 V for the sampling cone, and 4 V for the extraction cone; flow rate of desolvation gas (nitrogen) was equal to 500 l h^{-1} in ESI+ and 1000 l h^{-1} in ESI− mode.

3. RESULTS AND DISCUSSION

3.1. Identification of the substances released to the leachates

The release of any substances like additives or contaminants to deionised water from the studied bioplastics was tracked with the use of liquid chromatography coupled with electrospray ionisation mass spectrometry. Mass spectrometry methods are commonly used to detect the added substances in the bioplastics and their leachates (Gunaalana et al., 2020; Riboni et al., 2023). In this study the samples were analyzed both in the positive and negative ionisation modes. It was due to the fact that not all chemical substances can be detected in the individual ionisation mode. The respective total ion chromatograms were the outcome of this analysis.

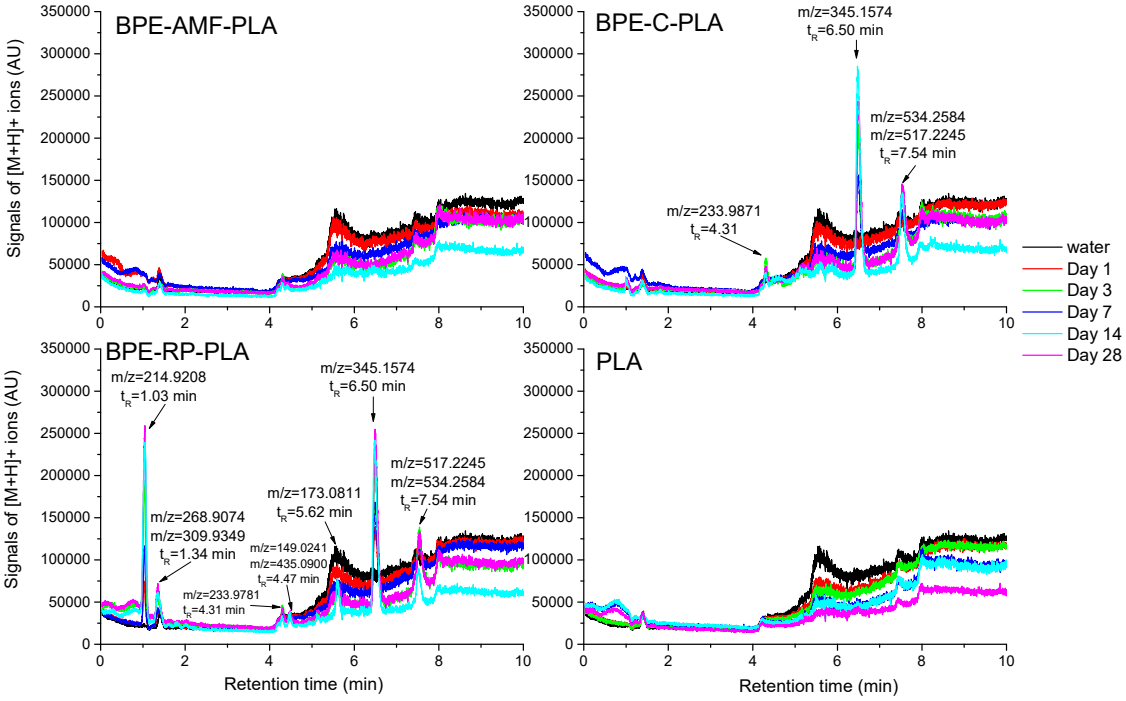

Figure 1. Positive ionisation mode total ion chromatograms of the leachates from the tested bioplastics.

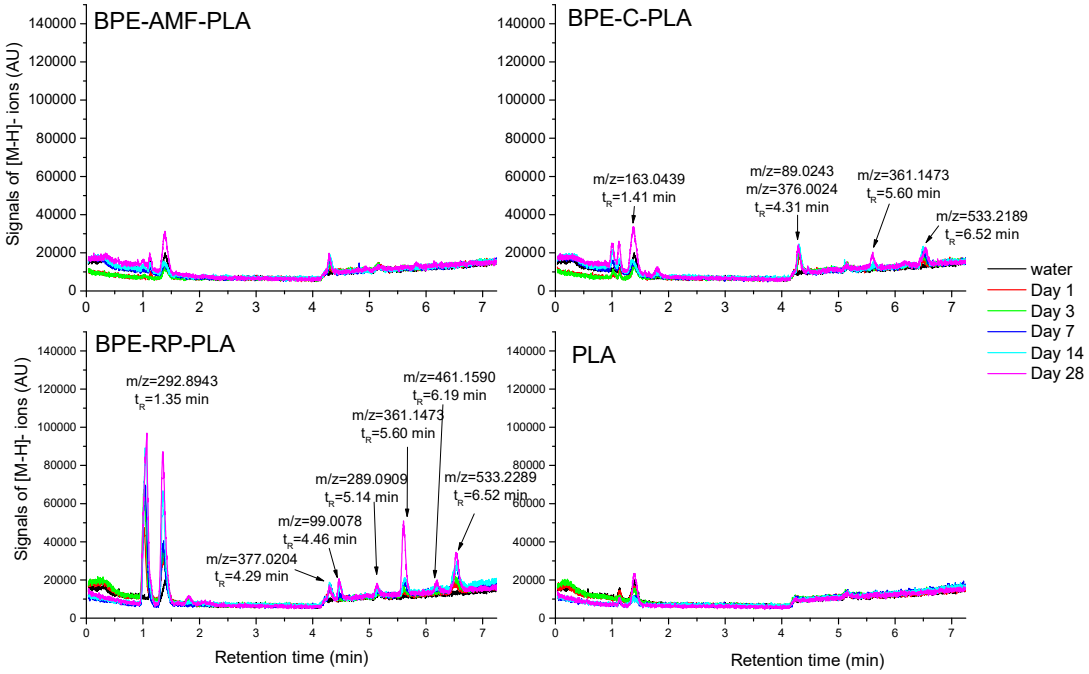

Figure 2. Negative ionisation mode total ion chromatograms of the leachates from the tested bioplastics.

It occurred in this study that chemical individuals released from the bioplastics were better ionised in the positive mode (stronger signals), but at the same time none of the ions detected were ionised in both modes under the mass spectrometry analytical conditions described in Materials and Methods section. Upon Table 1 it is impossible to find the respective negatively and positively ionised molecules, whose difference of m/z was equal to 2 masses of hydrogen atom. Thus 20 individual ions (10 in each ionisation mode) were found in the tested solutions either in the positive or negative ionisation modes. The individual ion was treated as found if the clear peak above the baseline of the total ion chromatogram was observed (Figs. 1 and 2).

Table.1. List of putative compounds released from the studied bioplastics.

No.	$(m/z)_{exp.}$	Retention time (min)	$(m/z)_{theor.}$	Absolute error $\Delta(m/z)= =(m/z)_{exp.}- (m/z)_{theor.}$	Assigned putative formula of the ion	Putative substance released	Bioplastic
Positive ionisation $[M+H]^+$							
1	214.9208	1.03					BPE-RP-PLA
2	268.9074	1.34					BPE-RP-PLA
3	309.9349	1.34					BPE-RP-PLA
4	233.9871	4.31					BPE-C-PLA, BPE-RP-PLA
5	149.0241	4.47	149.0239	+0.0002	$C_8H_5O_3$	Phthalic anhydride	BPE-RP-PLA
6	435.0900						BPE-RP-PLA
7	173.0811	5.62	173.0814	-0.0003	$C_8H_{13}O_4$	Diethyl fumarate	BPE-RP-PLA
8	345.1574	6.50	345.1603	-0.0029	$C_{22}H_{21}O_2N_2$	N,N-dibenzyl-terephthalamide	BPE-C-PLA, BPE-RP-PLA
9	534.2584	7.54					BPE-C-PLA, BPE-RP-PLA
10	517.2245						BPE-C-PLA, BPE-RP-PLA
Negative ionisation $[M-H]^-$							
1	292.8943	1.35					BPE-RP-PLA
2	163.1040	1.41	163.0330	+0.0071	$C_8H_8N_2S$	Methyl-2-mercapto-benzimidazole	BPE-C-PLA
3	377.0204	4.29					BPE-RP-PLA
4	89.0243	4.31	89.0239	+0.0004	$C_3H_5O_3$	Lactic acid	BPE-C-PLA
5	376.0024						BPE-C-PLA
6	99.0078	4.46	99.0082	-0.0004	$C_4H_3O_3$	Succinic anhydride	BPE-RP-PLA
7	289.0909	5.14					BPE-RP-PLA
8	361.1473	5.60					BPE-C-PLA, BPE-RP-PLA
9	461.1590	6.19					BPE-RP-PLA
10	533.2289	6.52					BPE-C-PLA, BPE-RP-PLA

Out of four tested microbioplastics, only in the case of BPE-C-PLA and BPE-RP-PLA any released molecules were detected as ions with the specific *m/z* values. Both for BPE-AMF-PLA and PLA no signals were found either in positive or negative ionisation modes (Figs. 1 and 2) as their total ion chromatograms hardly deviated from those of deionised water. In the case of pure PLA this result was not surprising as this polymer is prone to either acidic or alkaline hydrolysis (Wang et al., 2022). The highest number of the individual *m/z* values in the positive ionisation mode was detected for BPE-RP-PLA. These were 10 individual ions with measured *m/z* value (Fig. 1, Table 1). It must be clearly stated that the signal for *m/z* that equalled 345.1574 at the retention time of 6.5 min was the strongest for BPE-RP-PLA and for BPE-C-PLA, too. Also for BPE-RP-PLA at the early retention time the strong signal of the ion with *m/z* equal to 214.9208 was detected (Fig. 1). Furthermore, 4 out of these 10 individual masses were detected for BPE-C-PLA (Table 1).

Like in the positive ionisation mode for BPE-RP-PLA the highest number (equal to 7) of the individual ions was also detected in the negative mode, while for BPE-C-PLA these were 5 individual ions (Fig. 2). In the case of the negative ionisation mode only two specific ions were common for both BPE-C-PLA and BPPE-RP-PLA plastics, namely *m/z*=361.1473 and 533.2289.

Taking into account the time changes of the release of the chemical individuals from the bioplastics it was observed that in the case of ions detected in the positive ionisation mode e.g. those with *m/z* equal to 345.1574, 173.0811 and 214.9208 their signals were the highest already after 14 days. On the other hand some chemical individuals detected in the negative ionisation mode e.g. ions with *m/z* equal to 361.1473, 533.2289 and 292.8943 achieved their maximum signal on 28th day. It only proves different chemical nature of these substances but upon the knowledge of mass spectrometry it could not have anything common with ionisation mode used.

Upon the analysis of the total ion chromatograms it can be concluded that bioplastic BPE-RP-PLA had the lowest stability in the deionised water. Its chromatograms were full of detected ions, which must have contaminated the water phase above the plastic particles. In the case of BPE-C-PLA this contamination seems to be much lower than that of BPE-RP-PLA.

The identification of the detected ions to attribute them the chemical compounds was not an easy task. It was made on the level of putative identification only. Only 6 out of 20 detected ions could be attributed to a plausible chemical substance. Nevertheless, the probability of this identification was high, as the absolute error between the theoretical mass and detected mass in each case was lower that ±0.0080. Nevertheless it does not exclude misidentification

as there are compounds of the same molecular formula and different structures. The identification was made upon *m/z* values and the databases of chemical compounds like PubChem (pubchem.ncbi.nlm.nih.gov) and Metabolomics Workbench, (www.metabolomicsworkbench.org) and Plastic Additive Standards Guide. The mass spectra of the putatively identified compounds are shown in the Appendix from Fig. A1 to Fig. A7.

The ion of *m/z*=345.1574 that showed the highest signal in the positive ionisation mode for BPE-C-PLA and BPE-RP-PLA was most probably N,N-dibenzylterephthalamide (Fig. A3 and S4). This compound may be used as an additive to the plastics as according to Fries and Sühring (2023) various phthalates are on the list of suspects to be sought in the plastic leachates. The ion of *m/z*=149.0241 detected at BPE-RP-PLA might have been attributed to phthalic anhydride (Fig. A1) and the one of *m/z*=173.0811 released from the same bioplastic seemed to be diethyl fumarate (Fig. A2). Phthalic anhydride is known to be used in the production of plastics and bioplastics as a retarder. Retarders are substances added to plastics to allow for longer processing times and for the reduction of scorching. Regarding ions detected in the negative ionisation mode the m/z=163.0439 ion might have been attributed to methyl-2-mercaptobenzimidazole (Fig. A5). This substance is an accelerant, i.e. it increases the rate of the polymerization reaction or curing polymers. The ion m/z=89.0243 from BPE-C-PLA is likely to be lactic acid (Fig. A6) as polylactide plastics were studied. Nevertheless, one must be aware that there are two hydroxypropionic acids namely 2-hydroxypropionic acid and 3-hydroxypropionic acid. The first one is commonly called lactic acid. Finally, the ion of m/z=99.0078 might have belonged to succinic anhydride (Fig. A7) although such additive in BPE-RP-PLA is hardly plausible.

3.2. Effect of leachates on plant growth

Germination is regarded as a critical stage in higher plant growth and survival (Makhaye et al., 2021). In this study seed germination processes were affected neither in the experiments performed in the liquid phase (direct exposure) nor in the soil tests (Fig. 3). The best germinating plant was *Lepidium sativum*. All seeds of cress germinated in both the control tests as well as in the tests with bioplastic leachates (Fig. 3). The second dicotyledonous plant used as a model organism, i.e. *Sinapsis alba*, also germinated at very high percentage equal or exceeding 90% in the experiments with bioplastic leachates (Fig. 3). At the same time the mean germination percentage for this plant (SIA) in the control tests was 97% and 99% in the experiments made in the liquid phase and in the soil, respectively. Lower values of

germination percentage were found for the monocotyledonous plant sorghum. They varied from 77% to 100% in the liquid phase tests and from 77% to 93% in the soil tests (Fig. 3). Although in the case of sorghum the germination percentage was lower than that in the tests with dicotyledonous plants, it still remained at the high level according to the guidelines of Phytotoxkit Solid Samples (Microbiotests, Belgium). These guidelines indicate that the Phytotoxkit assay is valid if mean germination percentage is at least 70% for the control test. The prevailing view in the literature published so far has been that germination of higher plants was not susceptible to the effects of plastics/bioplastics (Balestri et al., 2019; Judy et al., 2019) or leachates from bioplastics, i.e. PHBV (Arcos-Hernandez et al., 2012).

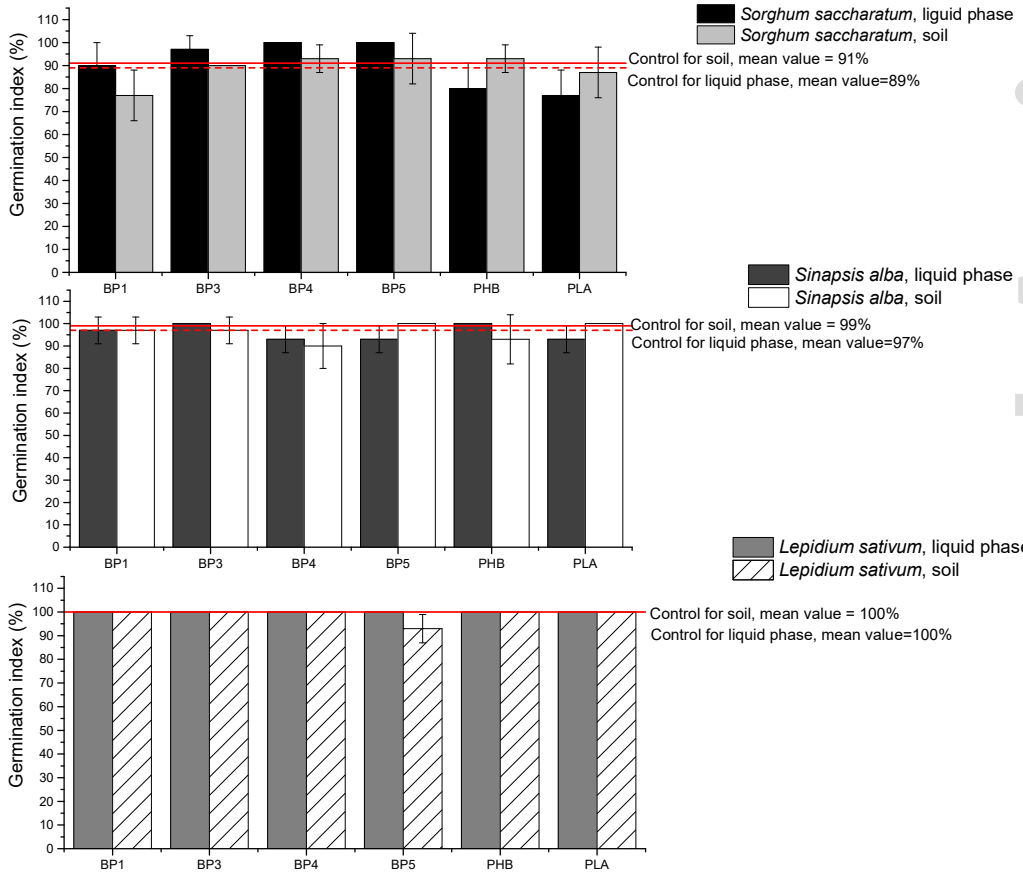

Figure 3. Effect of microbioplastic leachates on seed germination of higher plants.

The early growth of higher plants exposed to different types of microbioplastic leachates will be described for each model organisms studied in turn, starting with *S. saccharatum*. Both root and shoot length of sorghum in the liquid phase tests were lower than those in the control tests regarding the mean values (Fig. 4).

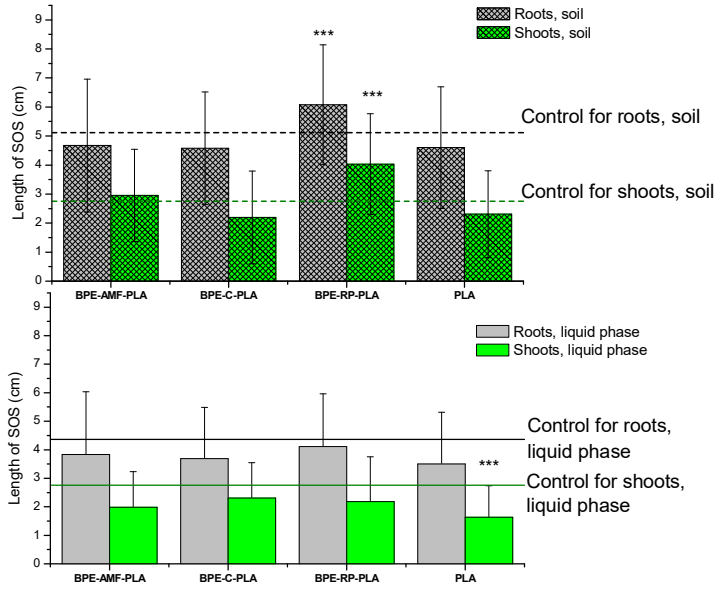

Figure 4. Length of roots and shoots of *S. saccharatum* exposed to microbioplastic leachates. The statistically significant difference between the samples and control was determined according to ANOVA test ($p \leq 0.05$) and marked by ***.

The reduction of root length exposed to microbioplastic leachates in comparison to the control runs was between 5.6% and 19.6% dependent on the bioplastic studied, while in the case of shoot length it was from 16.4% to 40.9%. Relating these calculations to the results of the statistical elaboration it was found that only for shoots of sorghum exposed directly to the leachates from PLA the reduction of their length in comparison to the control tests was statistically relevant ($p<0.05$) (Table 2a). At the same time in the soil tests with bioplastic leachates the root and shoot length of sorghum was usually lower than in the control tests with the exception for BPE-RP-PLA for both roots and shoots and BPE-AMF-PLA for shoots only (Fig. 4). In the soil tests the stimulation of growth of sorghum roots and shoots in the presence of leachates from BPE-RP-PLA was observed and confirmed statistically (Table 2b). The inhibition of early growth of sorghum exposed to the bioplastic leachates in the soil tests did not occur to be statistically relevant regardless of the bioplastic studied (Fig. 4, Table 2b).

Table 2a. Results for one-way ANOVA for all plants exposed to microbioplastic leachates directly in the liquid phase.

Tested compound	*p* - values					
	SOS (the liquid phase)		SIA (the liquid phase)		LES (the liquid phase)	
	roots	shoots	roots	shoots	roots	shoots
BPE-AMF_PLA	0.256	0.0550	0.513	0.104	0.266	0.00811 (S)
BPE-C-PLA	0.101	0.219	$9.214 \cdot 10^{-6}$ (S)	0.674	0.112	0.000102 (S)
BPE-RP-PLA	0.558	0.138	0.788	0.112	0.990	0.0303 (S)
PLA	0.0509	0.00405 (I)	0.0348 (S)	0.0131 (I)	0.126	0.000627 (S)

(I) - inhibition; (S) - stimulation

Table 2b. Results for one-way ANOVA for all plants exposed to microbioplastics in the soil.

Tested compound	*p* - values					
	SOS (the soil)		SIA (the soil)		LES (the soil)	
	roots	shoots	roots	shoots	roots	shoots
BPE-AMF_PLA	0.340	0.604	0.0000157 (I)	0.000799 (I)	0.00151 (I)	0.139
BPE-C-PLA	0.169	0.608	0.209	0.579	0.694	0.729
BPE-RP-PLA	0.0191 (S)	0.00100 (S)	0.903	0.0254 (I)	0.256	0.462
PLA	0.224	0.217	0.00000312 (I)	$1.295 \cdot 10^{-9}$ (I)	0.00275 (I)	0.477

(I) - inhibition; (S) - stimulation

The early growth of roots of mustard (SIA) in the liquid phase tests was not inhibited by the bioplastic leachates, whereas in the soil tests the inhibition occurred in the case of two bioplastics, BPE-AMF-PLA and PLA (Fig. 5). The reduction of root length was 35.4% in the soil tests with the leachates of BPE-AMF-PLA and 37.2% in the soil tests with the leachates of PLA (Fig. 5).The differences between the root length of mustard exposed and not exposed to the leachates of BPE-AMF-PLA or PLA were confirmed statistically ($p<0.05$) (Table 2b). With regard to the shoots of SIA, they were usually shorter in the tests with bioplastic leachates than in the control tests but it was observed particularly in the soil tests. The reduction of shoot length of mustard was found for the leachates from three bioplastics: BPE-

AMF-PLA, BPE-RP-PLA and PLA in the soil tests. It varied from 13.9% to 36.2% dependent on the bioplastic examined. The values of shoot length of mustard exposed and not exposed to bioplastic leachates in the soil were statistically different for these three bioplastics (Table 2). In the liquid phase tests the values of shoot length of mustard were at the same level as the ones in the control tests with the exception of leachates from PLA, for which they were by about 14.6% shorter than in the control tests (Fig. 5, Table 2a). At the same time Schiavo et al. (2020) observed the stimulation of root growth of *S. alba* by leachates from polypropylene (PP) in the liquid phase tests.

Regarding cress that was the second of dicotyledonous plants used as a model organism the early growth of roots was not affected in the direct exposure to the bioplastic leachates (the liquid phase tests), whereas in the soil tests the inhibition in the tests with the leachates of BPE-AMF-PLA or PLA was noticed. The difference between the length of the roots exposed and not exposed to the leachates of one of these two bioplastics was 10% for BPE-AMF-PLA and 8.5% for PLA (Fig. 6, Table 2b). At the same time the bioplastic leachates studied did not influence the growth of cress shoots in the soil tests (Fig. 6, Table 2b). In these tests the shoot length was at the same level in the tests with bioplastic leachates and in the control tests. In the liquid phase tests the stimulation of cress shoot growth was observed in each test regardless of the bioplastic studied. The shoots were longer in the liquid phase tests with bioplastic leachates than those in the control tests from 10.9% to 19.1% dependent on the bioplastic tested and these differences were statistically relevant (Fig. 6, Table 2a).

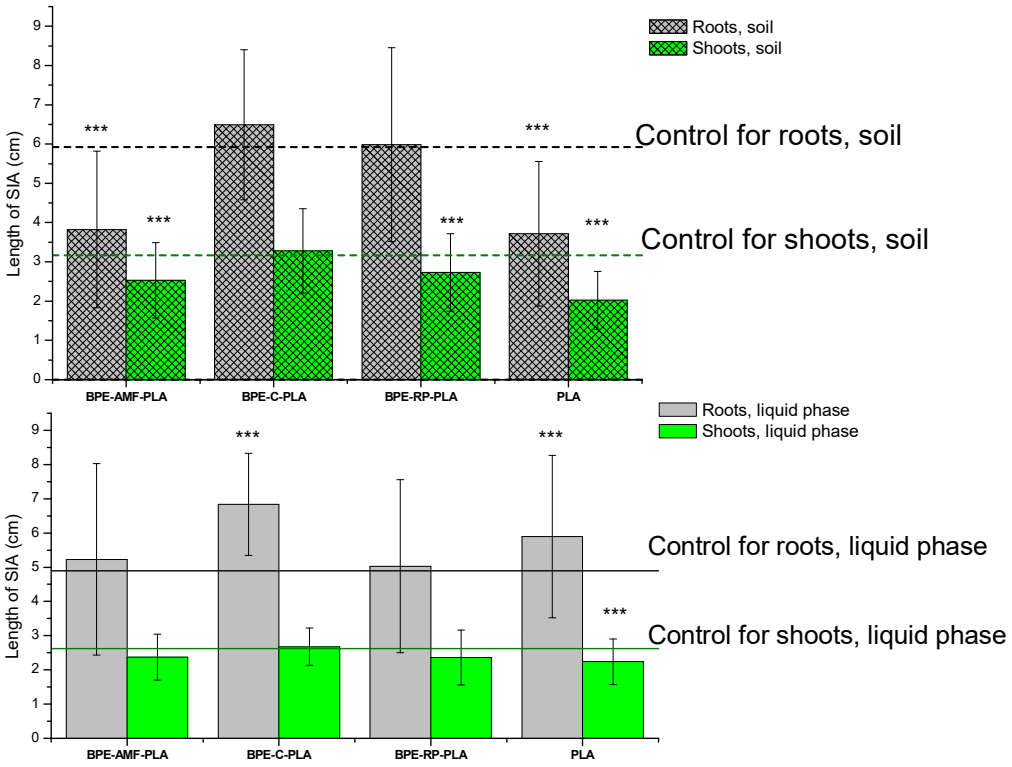

Figure 5. Length of roots and shoots of *S. alba* exposed to microbioplastic leachates. The statistically significant difference between the samples and control was determined according to ANOVA test ($p \leq 0.05$) and marked by ***.

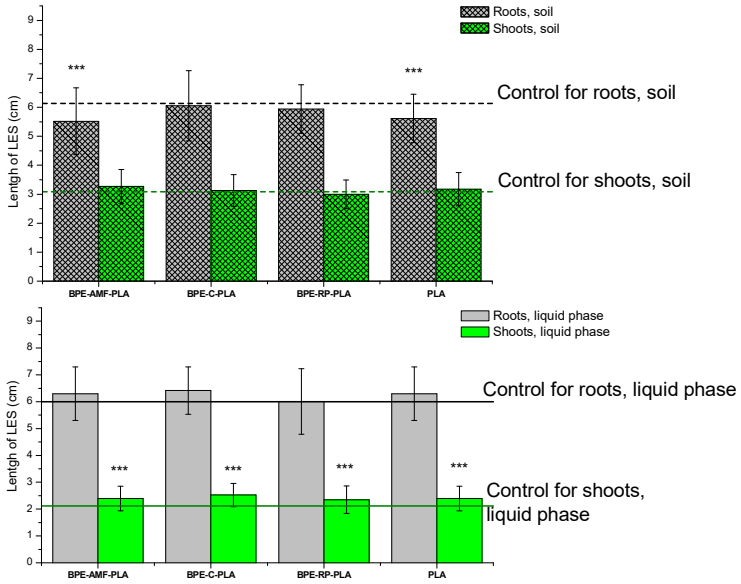

Figure 6. Length of roots and shoots of *L. sativum* exposed to microbioplastic leachates. The statistically significant difference between the samples and control was determined according to ANOVA test ($p \leq 0.05$) and marked by ***.

The obtained results of phytotoxicity tests showed that bioplastic leachates may act in different ways, i.e. no effect, inhibition, stimulation, or higher plant growth. The same was observed by Schiavo et al. (2020), who tested the influence of the leachates of three polymers polyethylene (PE), polypropylene (PP), polystyrene (PS) on the early growth of plants in the liquid phase tests. However, no toxic effect on plants dominated (Schiavo et al., 2020). Also in this study the growth of plants exposed to bioplastic leachates was usually unaffected considering the statistical confirmation as a criterion. Menicagli et al. (2019) made similar findings, as those presented in this work and by Schiavo et al. (2020), with regard to the interactions between leachates from HDPE or a new generation of compostable bags (MB) and growth of dune plants *Thinopyrum junceum* and *Glaucium flavum* used as model organisms. Bioplastic leachates exerted stronger effects on dicotyledonous plants than monocotyledonous ones that might have been connected with the differences in cell wall composition and structure (Henry and Harris, 1997; Schiavo et al., 2020).

4. CONCLUSIONS

1) Some studied bioplastics like BPE-C-PLA and BPE-RP-PLA are not chemically stable, as several ions that may be attributed to the individual chemicals, are released to deionised water after two weeks.

2) The compounds identified in the leachates from BPE-C-PLA and BPE-RP-PLA did not contribute to the phytotoxic effects either directly or in the soil matrix.

3) Dicotyledonous plants are more sensitive than monocotyledonous ones with regard to the evaluation of potential phytotoxicity of bioplastic leachates. *Sinapsis alba* occurred to be the most sensitive model organism.

4) The direct exposure to microbioplastic leachates contributes rather to the stimulation than to the inhibition of the early growth of plants. The stimulation, if occurs, concerns primarily shoot growth.

5) In the soil tests the inhibition of root and shoot growth of dicotyledonous plants occurs more frequently than in the liquid phase tests (the direct exposure). It indicates the potential interactions between the chemicals released to the leachates and soil matrix.

6) The leachates of BPE-AMF-PLA and PLA impact stronger on early growth of higher plants than the leachates of other bioplastics tested. They inhibit the growth of roots of dicotyledonous plants in the soil.

Summing up, the ecotoxicological evaluation of the effects of the bioplastics on the terrestrial ecosystems should comprise the ecotoxicity tests, in which the bioplastic leachates are studied with regard to their impact on the soil organisms. It is recommended because bioplastic leachates may contain chemicals affecting the biotic part of the terrestrial ecosystems.

Acknowledgements

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Appendix

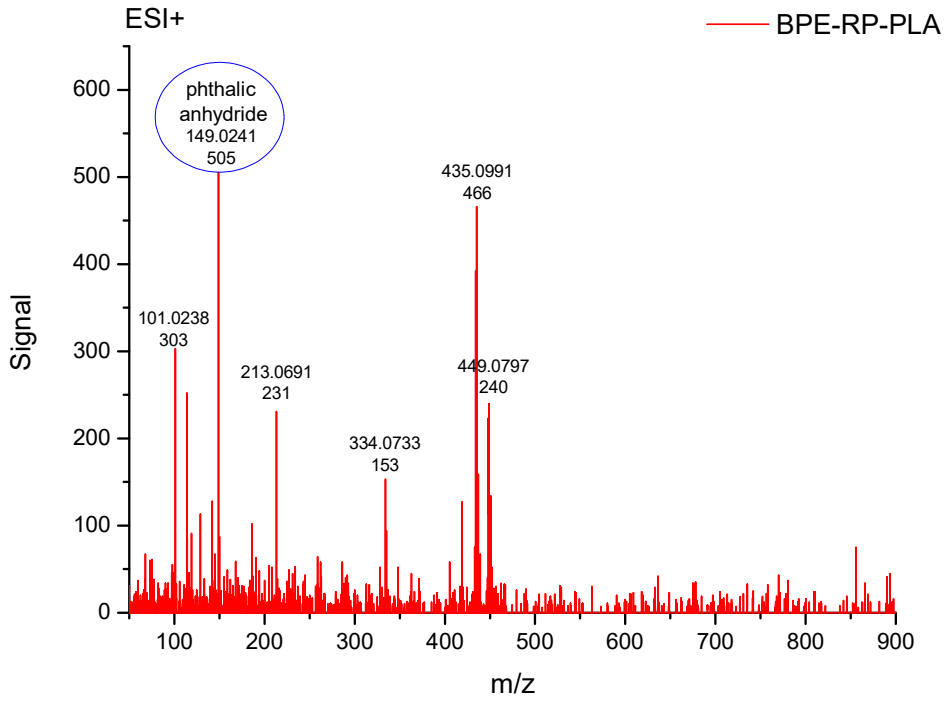

Figure A1. Mass spectrum to show phthalic anhydride released from BPE-RP-PLA.

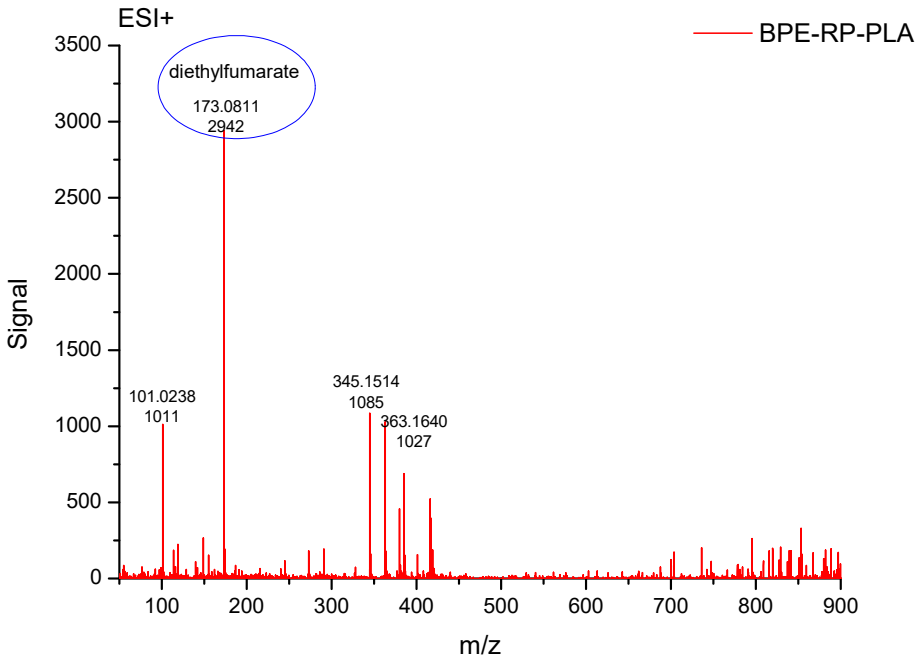

Figure A2. Mass spectrum to show diethylfumarate released from BPE-RP-PLA.

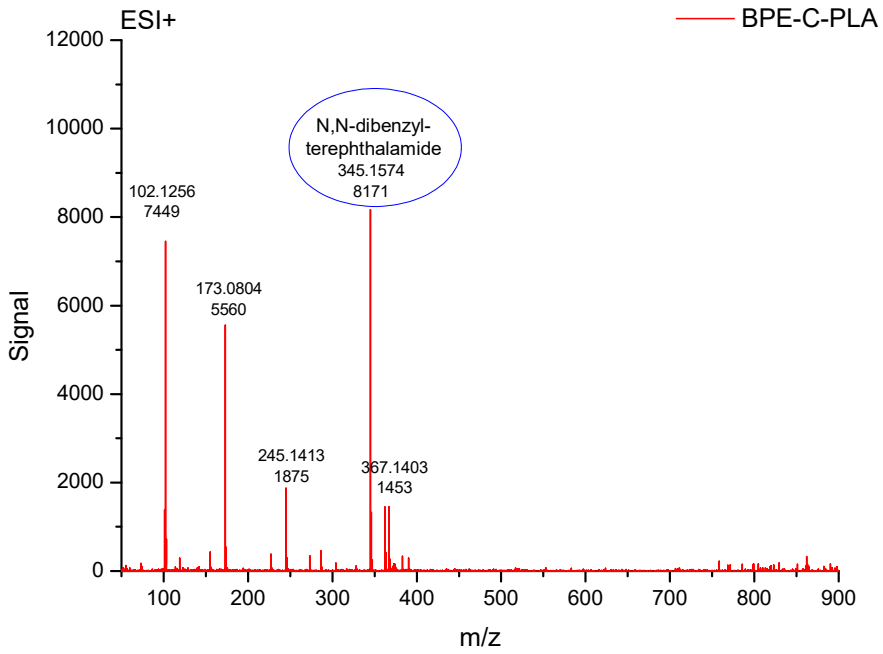

Figure A3. Mass spectrum to show N,N-dibenzylterephthalamide released from BPE-C-PLA.

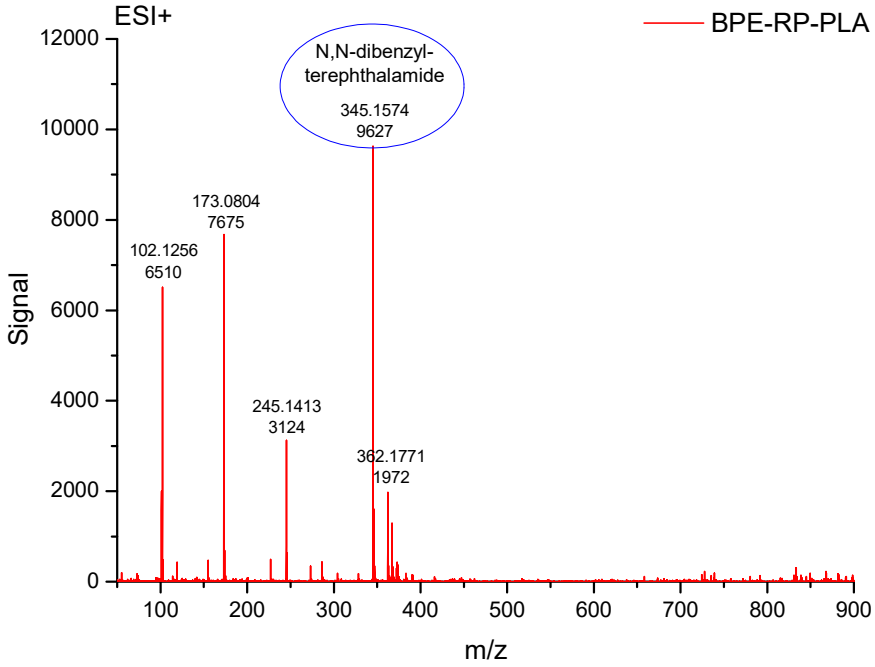

Figure A4. Mass spectrum to show N,N-dibenzylterephthalamide released from BPE-RP-PLA.

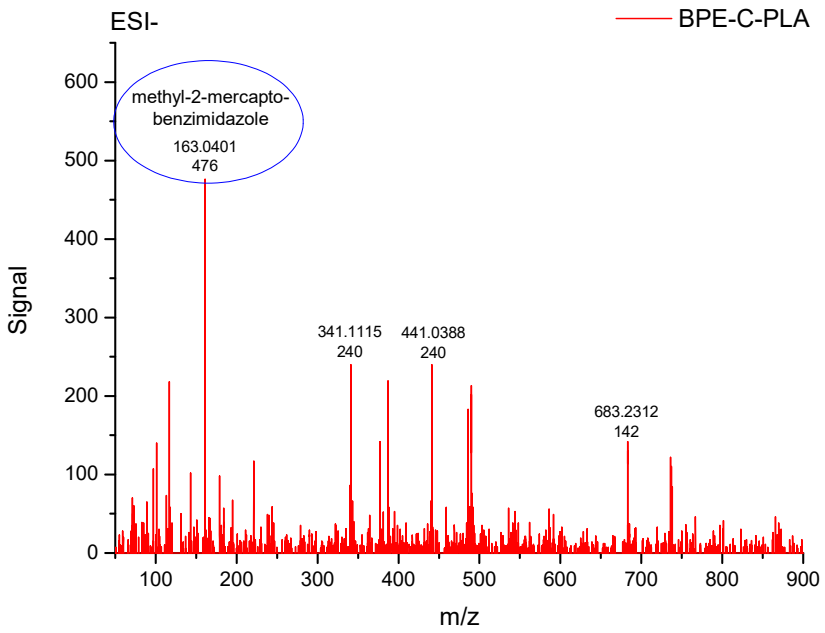

Figure A5. Mass spectrum to show methyl-2-mercaptobenzimidazole released from BPE-C-PLA.

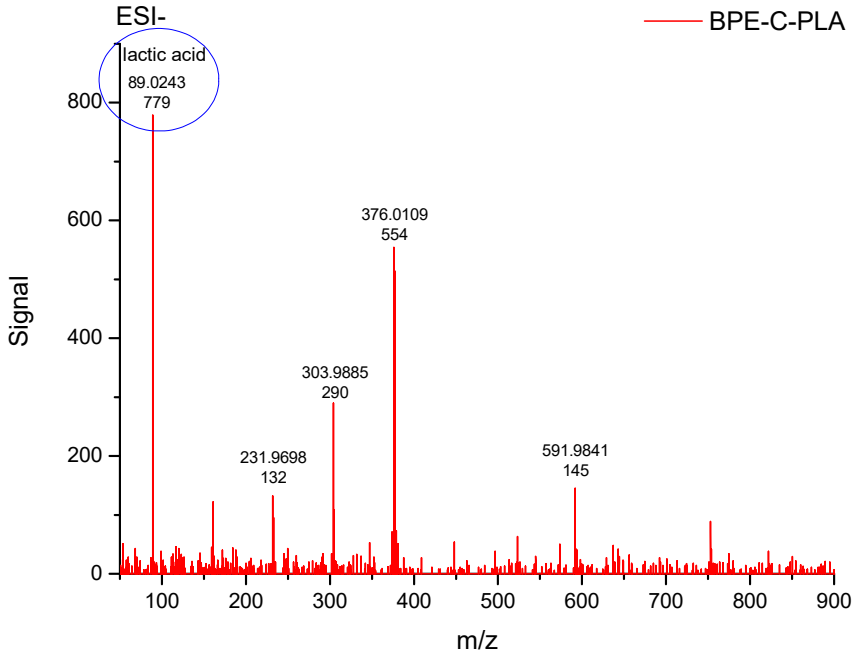

Figure A6. Mass spectrum to show lactic acid released from BPE-C-PLA.

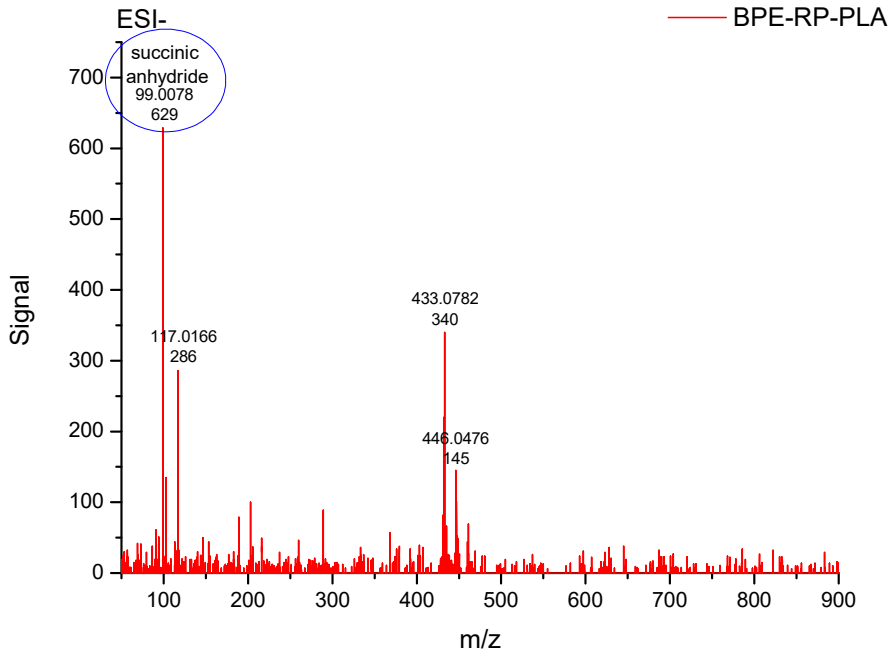

Figure A7. Mass spectrum to show succinic anhydride released from BPE-RP-PLA.