AppliedChem

MDPI

Article

Characterization of Potential Pollutants from Poly(lactic acid) after the Degradation Process in Soil under Simulated Environmental Conditions

Marta Krawczyk-Walach [1,*], Karolina Gzyra-Jagieła [1,2], Anna Milczarek [1] and Jagoda Jóźwik-Pruska [1]

[1] Łukasiewicz Research Network—Institute of Biopolymers and Chemical Fibres, 19/27 M. Skłodowskiej-Curie Street, 90-570 Łódź, Poland; k.jagiela@ibwch.lodz.pl (K.G.-J.); anna.milczarek@ibwch.lodz.pl (A.M.); j.jozwik-pruska@ibwch.lodz.pl (J.J.-P.)

[2] Faculty of Material Technologies and Textile Design, Lodz University of Technology, 116 Żeromskiego Street, 90-924 Łódź, Poland

* Correspondence: m.krawczyk-walach@ibwch.lodz.pl

Abstract: In recent years, the amount of produced petrochemical plastic waste has been growing at an alarming rate. According to the Plastics Europe Market Research Group (PEMRG)/Conversio Market & Strategy GmbH, in 2018 the global production of plastics amounts to 359 million tons, and in Europe—61.8 million tons. More than 80% of all marine litter is plastic, which accumulates in the environment due to its durability. Due to the growing problem, biodegradable polymer products are introduced to the market. Therefore, it is necessary to conduct research on degradation products in order to estimate the risk arising from their presence in the environment. This paper discusses research on compounds that may potentially remain in the soil after the degradation of the double green PLA polymer. The aim of the research was to prove whether products made of PLA, e.g., packaging, films and other waste can release substances harmful to the environment. Therefore, soil was selected as a medium to characterize the substances potentially released from the polymer under conditions simulating the degradation process in the environment. The soil was always used from the same producer. Before the polymer biodegradation process, it was additionally checked for pH, C and N content, number of microorganisms, etc. PLA degradation in soil was carried out in a laboratory accredited by the Polish Accreditation Center (PCA). During the research, soil samples at various stages of the degradation process under laboratory conditions were subjected to both extraction in an aqueous environment and organic solvent extraction The studies used the gas chromatography coupled with mass spectrometry (GC/MS), as well as pyrolysis gas chromatography (Py-GC/MS). In addition, the study used the gel permeation chromatography (GPC/SEC) allowing to determine the distribution of molar masses, average molar masses and polydispersity, and the infrared spectroscopy (FTIR).

Keywords: biodegradation; hydrolytic degradation; soil; extraction; PLA; GC/MS; Py-GC/MS; GPC/SEC; FTIR

Citation: Krawczyk-Walach, M.; Gzyra-Jagieła, K.; Milczarek, A.; Jóźwik-Pruska, J. Characterization of Potential Pollutants from Poly(lactic acid) after the Degradation Process in Soil under Simulated Environmental Conditions. *AppliedChem* **2021**, *1*, 156–172. https://doi.org/10.3390/appliedchem1020012

Academic Editor: Jason Love

Received: 30 October 2021
Accepted: 8 December 2021
Published: 10 December 2021

1. Introduction

Biodegradable polymers are increasingly used in various applications, such as packaging, textiles, medicine, and pharmacy. This is the result of the introduction of new legal regulations restricting the marketing of products made of polymers of petrochemical origin, e.g., EU Directive of 5 June 2019 No 2019/904 on the reduction of the impact of certain plastic products on the environment. Its main goal is to reduce the negative impact of some plastic products on the environment, in particular, on the aquatic environment and human health. It provides for the gradual phasing out of some plastic products, and where there is no alternative to the product, the introduction of extended producer responsibility to cover the costs of cleaning and managing plastic waste. The requirements of the directive

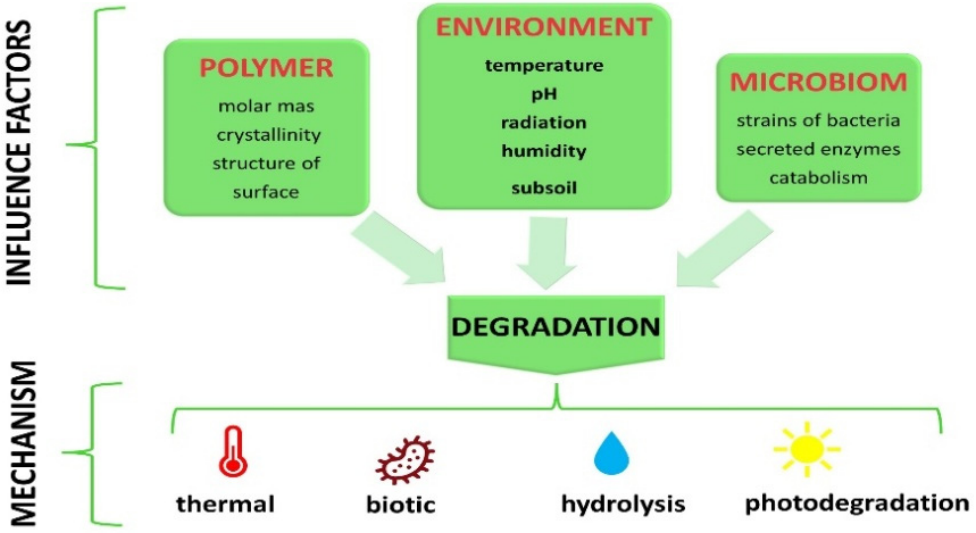
have forced the introduction of investments in the green technology sector. Their priority is a circular economy with innovative and sustainable business models, products, and materials [1]. The reduction of plastics in other regions of the world is also extremely important. Since 2011, India has banned the production and use of non-recyclable multi-layer plastic materials. African countries and a large part of Southeast Asia are introducing legal regulations to limit petrochemical plastics, especially single-use plastics [2,3].

In Europe, the introduction of the changes implemented in the Plastic Directive (Single-Use-Plastic) poses a considerable challenge for several entrepreneurs, which will most certainly affect the development strategy of many of them. Recently, there has been a tendency to replace traditional polymers with other materials that are degraded in the natural environment [4–8]. One such material that may offer an alternative for the future is poly(lactic acid)—PLA. This polymer belongs to the group of biodegradable linear aliphatic polyesters obtained from biomass; it shows both good physicomechanical and physicochemical properties [9–12]. Due to its favorable properties, such as biodegradability and good processability, it has now become an object of interest in the packaging industry [5,6,13–15]. PLA is obtained by polymerization of lactide (cyclic lactic acid dimer). There are three optical isomers of lactide: L-lactide, D-lactide, and DL-lactide. Therefore, it is possible to obtain various polymers: Poly [L-lactide], Poly [D-lactide], and Poly [DL-lactide]; the chemical properties of both chiral forms are similar to each other. The most frequently mentioned technique for obtaining PLA in the literature is ring-opening-polymerization [5,8,16–21].

Due to the growing problem of pollution of the natural environment with plastics, societal awareness increases, and hence the use of alternative materials that can be produced in a sustainable manner. Numerous studies based on the product life cycle confirm the advantage of PLA over polymers of petrochemical origin [22–24]. The use of PLA instead of conventional petrochemical polymers is a step in the right direction. PLA-based materials will degrade when exposed to various environmental conditions. Irreversible changes in the polymer due to degradation lead to a loss of properties. PLA can be degraded naturally by the simple hydrolysis of ester bonds when exposed to the appropriate conditions, which consist of a combination of moisture, oxygen, and naturally occurring microorganisms, PLA will break down and form low molecular mass metabolic products. Ultimately, they should decompose into water, carbon dioxide, and a small amount of non-toxic surplus material. Unfortunately, scientists have noticed that PLA-based materials are persistent in soil at ambient temperatures, which can cause significant environmental pollution, leading to similar problems of conventional fossil fuel-based plastics. Therefore, research is needed on the degradation of PLA and the related effective approaches to post-consumer waste management [8,16,19,21].

The degradation rate of PLA is influenced by many factors, including the isomer ratio, temperature, pH, burial time, humidity, oxygen, and the shape and size of the material (Scheme 1). The literature mentions several possible mechanisms of PLA degradation (hydrolytic, oxidative, thermal, microbiological, enzymatic, chemical, and photodegradable), which mainly cause cracks in the main and side chains. The mechanisms of enzymatic and microbiological degradation are of particular interest. These processes are faster and lead to the decomposition of PLA to CO_2 and H_2O. It should be emphasized that this subject is complicated, deeming it necessary to conduct research on the effects of the long-term impact of large amounts of PLA waste in the environment [8,16,19,21]. The degradation of various compounds, including polymers, in the soil environment is mostly slower than in compost. It results from the conditions of this medium (temperature, pH, and humidity), a different amount and structure of microorganisms, and other catabolic processes [8,25,26]. However, it remains a very rich environment in which there are various compounds derived from the metabolism of plants and animals e.g., plant secondary compounds (PSCs), terpenes, flavonoids, glucosinolates, and alkaloids, and many different chemical structures containing carbon, nitrogen, phosphorus, and sulfur [27,28]. Unfortunately, the soil also contains pollutants from the human economy. In the Global assessment of soil

pollution—Summary for policy makers report, many different soil polluting compounds and their impact on the environment and human health were indicated [29]. PLA is also used in biomaterials in the medical and pharmaceutical sector, e.g., as implants, due to degradation [30,31]. In body fluids, the process is different than in the environment, i.e., soil, compost.



Scheme 1. PLA degradation mechanisms and factors influencing this process.

The aim of the research was to develop a methodology for the determination of compounds that may potentially remain in the soil as a result of PLA decomposition. Soil samples, at various stages of the biodegradation process, were subjected to both extractions: in the water environment and organic solvent extraction. The studies used the classical technique of gas chromatography coupled with single quadrupole mass spectrometry (GC/MS) as well as the pyrolysis gas chromatography (Py-GC/MS). This technique is used in the analysis of polymers; this solution enables the analytes to be released as a result of pyrolysis and introduced directly into the chromatography column without any losses. In addition, the study used the technique of gel permeation chromatography (GPC/SEC) allowing to determine the molar mass distribution, average molar masses, and polydispersity. The technique of infrared spectrophotometry (FTIR) was also used, which allows for the study of the structure of polymers to cover part of the electromagnetic spectrum in the range 4000–625 cm^{-1}. Using this technique, the chemical properties of the material and the functional groups present can be determined.

2. Materials and Methods

2.1. Chemical and Reagents

Poly (D,L-lactide) 6201D (PLA) was purchased from NatureWorks® LLC (Minnetonka, MN, USA); Chloroform stabilized with Amylene, HPLC grade was purchased from Chem-Lab NV (Zedelgem, Belgium); N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) and N,O-Bis (trimethylsilyl) acetamide (BSA)—GC derivatization reagents; 3,6-Dimethyl-1,4-dioxane-2,5-dione (lactide) were purchased from Sigma–Aldrich (Saint Louis, MO, USA), lactic acid 88% was purchased from POCh (Gliwice, Poland); Acetonitrile HPLC (MeCN) was purchased from Chemsolve-WITKO (Łódź, Poland), anhydrous sodium sulfate (dried at 500 °C for 3 h) was purchased from J.T. BAKER (Phillipsburg, NJ, USA).

The PLA solution was prepared by dissolving one granule in 10 mL of chloroform (final concentration 3.8 g/dm^3). Stock standard solutions were prepared by dissolving an appropriate amount of lactide (final concentration 20 g/dm^3), and lactic acid (final concentration 1% or 0.01%) in MeCN. Stock solutions were kept at 4 °C for several days without

noticeable change of content. The working solutions were prepared by dilution with MeCN as needed. All reagents were tested and found to be stable for unattended analysis.

2.2. Biodegradation and Sample Collection

The tests of biodegradability (microbiological decomposition) were performed in the soil environment in accordance with an accredited procedure based on international standards (PN-EN ISO 11266: 2020-11; PN-EN ISO 11721-1: 2002; PN-EN ISO 11721-2: 2005). The soil is always used from the same producer from Poland. Before the polymer biodegradation process, it was additionally checked for pH, C and N content, number of microorganisms etc. PLA degradation in soil was carried out in a laboratory accredited by the Polish Accreditation Center (PCA). Due to the PCA supervision, the selected soil should always have specific parameters so that the process was repeatable. A specific type of soil was entered in the test procedure based on normative documents. The research involved a granulate of poly (D, L-lactide). The biodegradation studies were carried out under aerobic conditions in the soil environment under controlled temperature and humidity conditions (30 ± 2 °C; 80%) simulating the natural processes occurring during decomposition. The humidity control was performed daily. The medium used in the research was not fertilized. Before starting the tests, the microbiological activity of the test soil and its humidity were determined (≥106 cfu/mL; 75.8%). The biodegradation process was monitored by removing individual samples from the inoculum at specified intervals, washing and drying to the constant weight. Then the relative weight loss was determined. According to international standards [32], the tests were conducted for a period of 24 weeks. It is assumed that during this period for biodegradable materials, the loss of mass should not be less than 90%. GC/MS, Py-GC/MS, GPC/SEC, and FTIR analysis for blank soil samples (G0), samples after 20 weeks (G20) and 24 weeks (G24) of the biodegradation process were performed.

2.3. Recommended Analytical Procedure

2.3.1. Preparation of Soil Extracts

Two extraction media to prepare the material for testing were used. Soil samples were extracted with chloroform due to the fact that PLA is a compound soluble in this solvent. The second medium was water because degradation in the environment occurs with the participation of this solvent.

For the preparation of the organic chloroform HPLC extract, 1 g (±0.01 g) of soil was obtained and carried out in two parallel ways:

(a) extraction in an ultrasonic bath for 1 h (temperature ~30 °C)—this type of extraction is becoming more and more popular among researchers around the world, it allows to significantly shorten the time of sample preparation with high recoveries at the same time;
(b) cold extraction—a sample placed in a solvent for 24 h (temperature ~23 °C).

After extraction, the samples were vacuum filtered on a Whatman three-part funnel using Whatman GF/D 47 mm glass fiber filters (Maidstone, UK). Each time the residue was washed with an additional portion of chloroform, to minimize the loss of analytes. The next step was drying the samples with anhydrous sodium sulfate and concentrating them in a vacuum evaporator to the volume of 1.5 mL. If necessary, the samples were concentrated under a stream of nitrogen. In order to remove possible residual fine impurities, the concentrated extracts were filtered through 0.25 mm RC syringe filters with a pore diameter of 0.45 µm (Sartorius, Getynga, Germany) before being transferred to the chromatographic vials.

Due to the fact that in the natural environment the degradation process does not occur with the participation of organic solvents but with the participation of water, the next stage of the research was to carry out an extraction in an aqueous environment. As described above, the same amount of soil 1 g (±0.01 g) was used. The water extraction with purified water was carried out in two ways: (a) in an ultrasonic bath—1 h, temperature ~30 °C, (b) cold water—24 h, temperature ~23 °C. After extraction, the samples were filtered on

a three-part Whatman funnel under vacuum using GF/D 47 mm glass fiber filters of the same company. Each time the residue was washed with additional water to minimize loss of analytes. The next step was to extract the filtrate with chloroform in a separating funnel. The GC/MS and GPC/SEC techniques require the preparation of the sample in an organic solvent; therefore, this step was necessary. Due to the fact that PLA dissolves in chloroform, this compound readily penetrates from the aqueous to the organic phase. Extraction was performed for 5 min; after separation, the chloroform phase was collected in a conical flask and dried with anhydrous Na_2SO_4. The extracts were concentrated to 1.5 mL in a vacuum evaporator. The rest of the steps (concentration under a stream of nitrogen and filtration with syringe filters) were the same as for the solvent extraction.

2.3.2. GC/MS and Py-GC/MS Analysis

Soil samples and their extracts prepared according to the procedures presented in Section 2.3.1, as well as the PLA granules, lactide and lactic acid stock solutions were subjected to chromatographic analyzes. Two techniques of gas chromatography were used in the research: classical gas chromatography coupled with mass spectrometry (GC/MS) and the technique of pyrolysis gas chromatography coupled with mass spectrometry (Py-GC/MS). The analysis were carried out using a gas chromatograph 7890B from Agilent Technologies (Santa Clara, CA, USA) coupled with a single quadrupole 5977B MSD mass spectrometer of the same company. Samples were separated on HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Our GC/MS is equipped with an additional GERSTEL (Mulheim, Germany) module (TDU) for pyrolysis and/or thermal desorption of the sample, which is an integral part of the equipment.

(1) GC/MS Analysis

The GC/MS technique allowed for the preliminary development of a method for determining the main decomposition products of PLA: lactide and lactic acid. Lactide standard solution (20 g/dm^3) was diluted to a concentration of 0.1 g/dm^3. The prepared sample was subjected to chromatographic analysis using the chromatograph operating parameters presented below. The column temperature was initially held at 40 °C for 2 min, then the temperature was raised to 300 °C at a rate of 10 °C per min and held for 10 min. Helium purity 6.0 from Linde Gaz (Kraków, Poland) was used as carrier gas. The carrier gas flow rate was 1 mL/min. Injector temperature was maintained at 250 °C, and the injection volume was 1.0 µL in the splitless mode. The ion source temperature held at 280 °C. Mass spectra were scanned from m/z 10–650 at a rate of 1.5 scans/s. Electron impact ionization energy was 70 eV.

Determination of lactide retention time (11.089 min) allowed to improve the qualitative analyzes of the studied soil extracts. Figure 1 shows a representative chromatogram for the lactide sample at 0.1 g/dm^3.

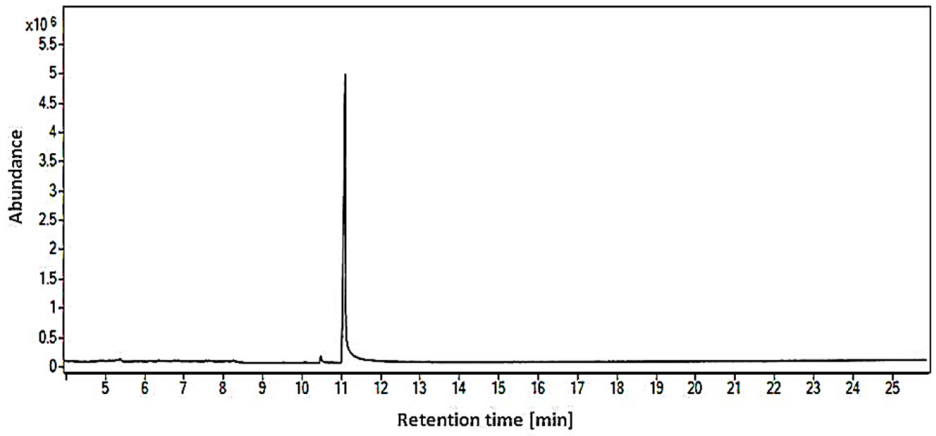


Figure 1. A representative chromatogram obtained for lactide sample [0.1 g/dm^3].

For the determination of lactic acid by GC/MS, a derivatization reaction is needed. Two commercially available derivatizing reagents were used in the research: BSTFA and BSA. Lactic acid (88%) was diluted in MeCN to concentration 0.01%. Chromatographic analyzes were carried out using the same parameters of the apparatus operation as for as lactide. Specified volumes of BSA (2–50 µL) or BSTFA (1–20 µL) were added to 0.01% lactic acid solutions. The samples were thoroughly mixed and left for at least 30 min for the silylation reaction to proceed. Both derivatization reagents formed a stable derivative with lactic acid, but higher reaction yields occurred with BSTFA. Chromatographic analyzes were performed in the SCAN mode for counting the mass fragments m/z 10–650, which allowed for the determination of lactic acid retention time (9.416 min). Determination of lactic acid retention time allowed to improve the qualitative analyzes of the studied soil extracts.

The next step during the research was the performance of chromatographic analyzes for the prepared samples of soil extracts. Due to the fact that lactic acid may be one of the degradation compounds of PLA, it was necessary to carry out the derivatization of the extracts. 4.5 µL of BSTFA was added to 150 µL of the sample. It was shaken vigorously and left for 30 min. The samples prepared in this way were subjected to GC/MS analysis with the use of the apparatus operating parameters as in the case of lactide and lactic acid determination.

(2) Py-GC/MS Analysis

The Py-GC/MS differs from the classic GC/MS in terms of sample preparation. It is a thermal analysis technique and consists in placing the tested material in a pyrolysis test tube, heating it to a properly selected temperature and chromatographic analysis of the products of combustion/degradation of the sample. A sample of PLA or soil (approx. 0.200 µg) was introduced into the pyrolysis tubes. The solutions (1 µL) placed on the burnt-out glass wool in the pyrolysis tube were also analyzed. The parameters of the apparatus operation used during soil analyzes are presented below. Samples were pyrolyzed at 400 °C for 0.08 min. The TDU temperature was set from 50 °C to 300 °C with a heating rate of 100 °C/min. CIS injector temperature was maintained at 300 °C, and the injection volume was 1.0 µL in the splitless mode. The pyrolysis products were immediately subjected to chromatographic analysis. The column temperature was initially held at 40 °C for 2 min, then the temperature was raised to 300 °C at a rate of 10 °C per min and held for 10 min. Helium purity 6.0 from Linde Gaz (Kraków, Poland) was used as carrier gas. The carrier gas flow rate was 1 mL/min. The ion source temperature held at 280 °C. Mass spectra were scanned from 10–650 m/z at a rate of 1.5 scans/s. Electron impact ionization energy was 70 eV.

2.3.3. Molar Mass Distribution and Polydispersity

Molar mass distribution and polydispersity of samples were analyzed by gel permeation chromatography/size exclusion chromatography (GPC/SEC) method. The tests were performed using 1260 Infinity system (Agilent Technologies, Santa Clara, CA, USA) with an Optilab refractometric detector (Wyatt Technology, Goleta, CA, USA). The average molar weights were determined using the conventional calibration technique. The tests were performed using chloroform as the eluent and one PLgel Mixed-C 300 × 7.5 mm chromatographic columns (Agilent Technologies, Santa Clara, CA, USA) at a flow rate of 0.7 mL/min.

2.3.4. Fourier Transform Infrared Spectroscopy

Transmission spectra were obtained using the Nicolet iS50 infrared spectroscopy (Thermo Scientific, Waltham, MA, USA), the operating parameters were as follows: measurement range 4000–625 cm^{-1}, resolution 4.0 cm^{-1} and the number of scans for baseline and spectrum collection 32. The accuracy of wavenumbers reading for characteristic bands was ± 1 cm^{-1}. The soil after chloroform extraction were subjected to spectroscopic examination. Soil samples before the start of the biodegradation process and after 20 and

24 weeks of biodegradation (G0, G20, G24) as well as extracts of three commercial blank soils were tested. Infrared spectroscopic analyzes were also performed for PLA standard solutions in $CHCl_3$ (concentration 3.8 g/dm^3), lactide in MeCN (concentration 1 g/dm^3) and lactic acid in MeCN (concentration 1%). Samples for tests (20 µL) were applied to the NaCl window. After evaporation, spectroscopic analyses of the solvent were performed.

3. Results and Discussion

3.1. Soil Analysis after Solvent Extraction

3.1.1. GC/MS Analysis

The studies performed by GC/MS technique suggest that in the case of soil samples after extraction, both in an ultrasonic bath and in a cold extraction for 24 h in chloroform (Figures 2 and 3), enrichment of the matrix along with the degradation time was observed. For these experiments, the presence of lactide and lactic acid after 24 weeks of the degradation process was observed. Samples G0 and G20 did not contain these compounds, or only in trace amounts. For soil samples after extraction in water (Figures 4 and 5), peaks from lactic acid and lactide in samples after 24 weeks of the PLA degradation process were observed. After 24 weeks, no weight loss of sample or macroscopic changes in PLA after the biodegradation process were observed. However, after this time, the GC/MS results show the presence of low molecular mass compounds, which were not present in the soil blank extract. This may indicate degradation of the polymer.

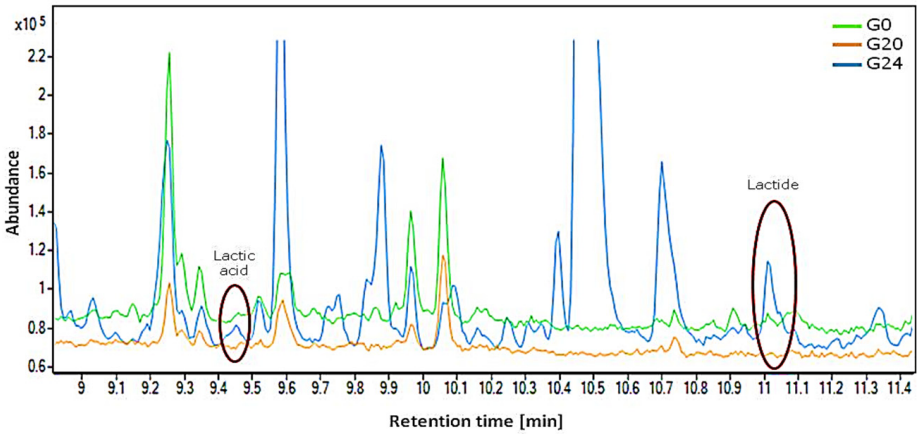


Figure 2. Chromatograms obtained for soils after chloroform extraction using an ultrasonic bath (G0-soil before degradation, G20-soil after 20 weeks of degradation, and G24-soil after 24 weeks of degradation).

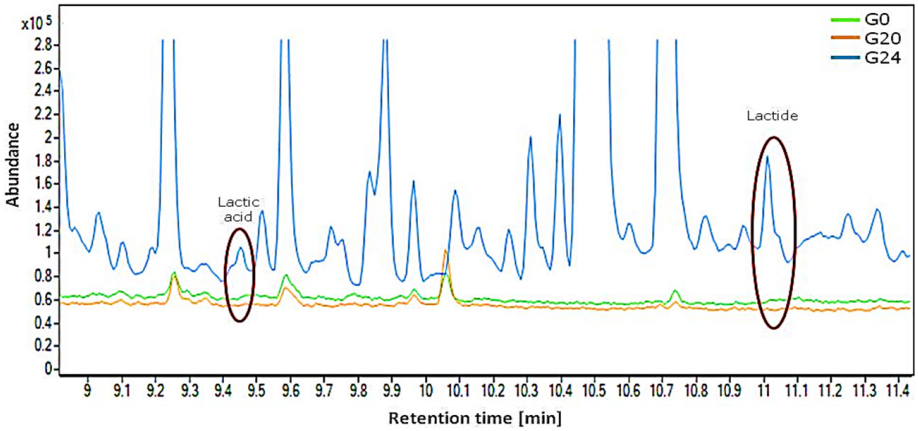


Figure 3. Chromatograms obtained for soils after cold extraction in chloroform for 24 h (G0-soil before degradation, G20-soil after 20 weeks of degradation, and G24-soil after 24 weeks of degradation).

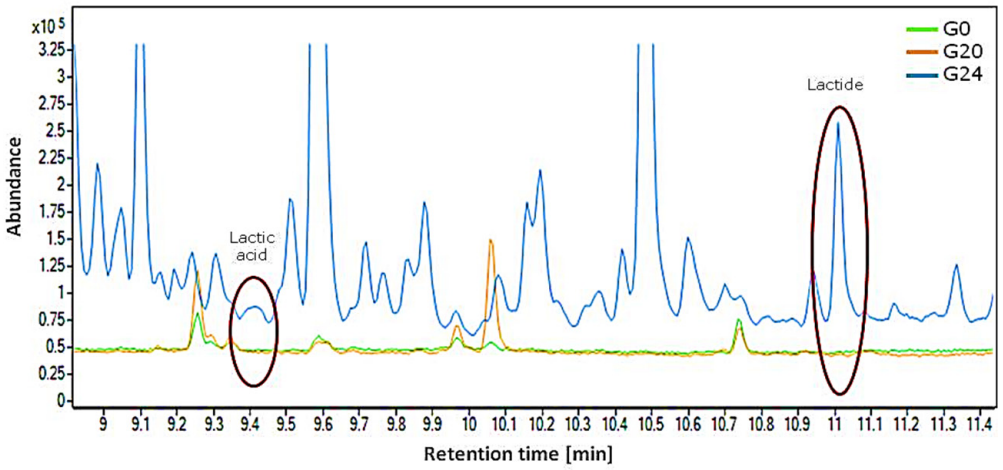


Figure 4. Chromatograms obtained for soils after water extraction using an ultrasonic bath (G0-soil before degradation, G20-soil after 20 weeks of degradation, and G24-soil after 24 weeks of degradation).

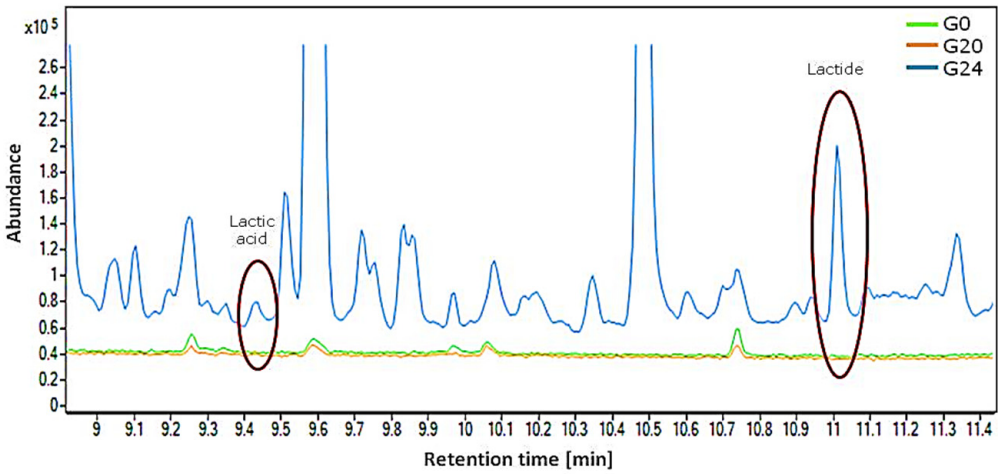


Figure 5. Chromatograms obtained for soils after cold extraction in water for 24 h (G0-soil before degradation, G20-soil after 20 weeks of degradation, and G24-soil after 24 weeks of degradation).

3.1.2. GPC/SEC Analysis

Tests of aqueous or chloroform soil extracts in order to determine the molar mass, polydispersity, and distribution of molar mass structures were carried out. The results are presented in Table 1 and Figures 6–8. The peaks of the extracted structures slightly overlap with those of the eluent. For each type of extraction in blank soil sample, the structures with low values of molar mass, both extracted with chloroform and water, were determined. In the case of direct extraction with an organic solvent, the values were slightly higher, approximately 1000 g/mol, and after extraction in water, approximately 700 g/mol. After the degradation process, structures with higher values were also found and this value increases as a function of time.

Depending on the extraction medium used, different MMD distributions were obtained (Figures 6–8). For extraction with chloroform, the mean values of molar masses were higher and the mass distribution was in a wider range, which is also evidenced by the higher values of polydispersity. The obtained results showed the complexity of the blank soil samples, but the presence of PLA-derived structures in the blank soil samples was surprising and disturbing. As a result of the degradation process after extraction, both in chloroform and in water, an increase in mean molar mass values (M_w) and a much wider range of MMD can be noticed as a function of time. The extracted structures were more polydisperse; however, no inference can be made about their origin. It is not possible to

state unequivocally whether these were PLA degradation products (secondary structures) because there were similar structures in blank soil samples (primary structures). In the blank soil sample, low molar mass compounds e.g., lactic acid, lactide by GC-MS analysis were not determined. Soil is an environment rich in various compounds of natural (plant, animal) or external origin (pollution); therefore, larger molar mass compounds soluble in water and chloroform in blank soil were detected by GPC/SEC analysis. After 24 weeks of degradation of PLA structures with higher M_w, which can potentially come from polymer degradation, were observed.

Table 1. Results obtained for structures after extraction from soil.

Sample	Medium	Extraction Type	Time of Degradation, Week	M_n, g/mol per PS	M_w, g/mol per PS	(M_w/M_n)
G0 $_{\text{Chloroform 24}}$	$CHCl_3$	24 h	0	380	900	2.4
G20 $_{\text{Chloroform 24}}$	$CHCl_3$	24 h	20	500	930	1.9
G24 $_{\text{Chloroform 24}}$	$CHCl_3$	24 h	24	590	1260	2.1
G0 $_{\text{Chloroform Ult}}$	$CHCl_3$	ultrasonic	0	500	1010	2.0
G20 $_{\text{Chloroform Ult}}$	$CHCl_3$	ultrasonic	20	520	1170	2.3
G25 $_{\text{Chloroform Ult}}$	$CHCl_3$	ultrasonic	24	570	1440	2.5
G0 $_{\text{Water 24}}$	H_2O	24 h	0	520	780	1.5
G20 $_{\text{Water 24}}$	H_2O	24 h	20	550	1190	2.1
G24 $_{\text{Water 24}}$	H_2O	24 h	24	420	1170	2.8
G0 $_{\text{Water Ult}}$	H_2O	ultrasonic	0	480	650	1.4
G20 $_{\text{Water Ult}}$	H_2O	ultrasonic	20	480	840	1.7
G24 $_{\text{Water Ult}}$	H_2O	ultrasonic	24	390	1060	2.7

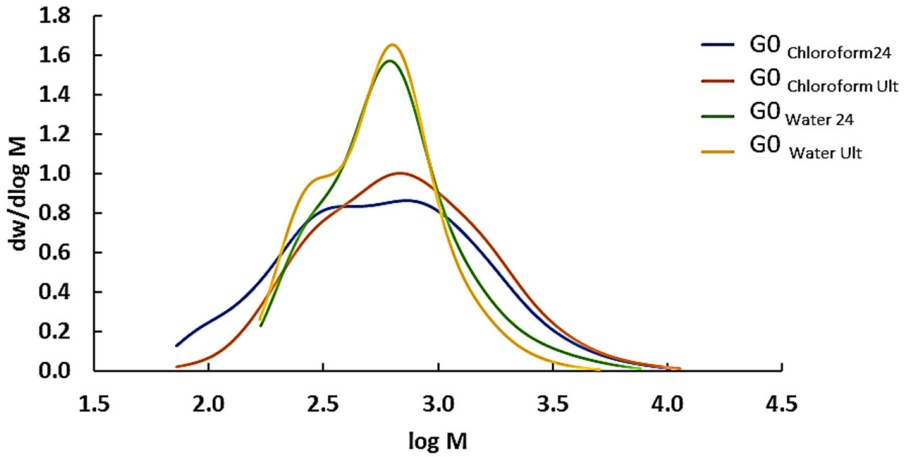


Figure 6. Molar mass distribution of structures in soil extracts before the degradation process.

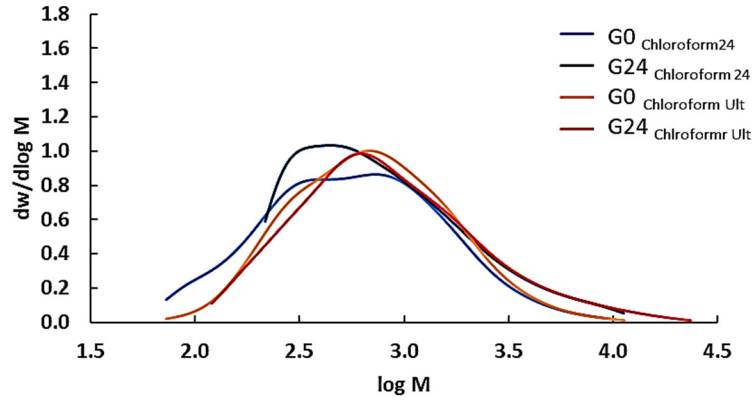


Figure 7. Molar mass distribution of structures in soil after the degradation process, 24 weeks after extraction with chloroform.

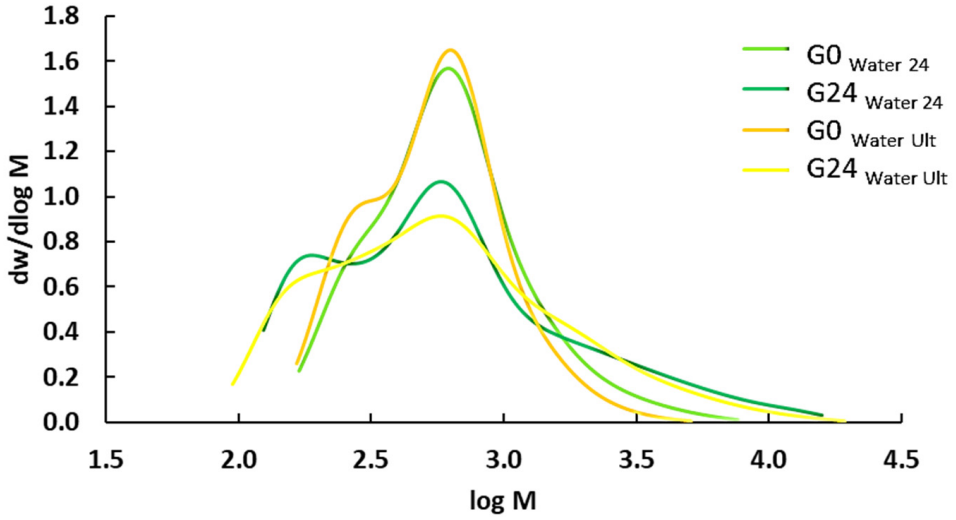


Figure 8. Molecular mass distribution of structures in soil after the degradation process following 24 weeks of water extraction.

3.1.3. FTIR-Analysis

The infrared spectroscopy (FTIR) to study changes in the structure of the tested polymer during the PLA degradation in soil was used. The tests on the PLA standard solution (3.8 g/dm^3) in chloroform, as well as the standard solution of lactide (1 g/dm^3) in MeCN and lactic acid (1%) in MeCN, which may be one of the possible intermediates of the polymer degradation, were carried out. Figures 9–11 show the IR spectra obtained for PLA, lactide and lactic acid. The transmission spectrum of PLA contained characteristic groups for this polymer (Figure 9), i.e., band corresponding to the carbonyl groups of the ester (1756 cm^{-1}), bands stretching asymmetric and symmetric C-H bonds: 2995 cm^{-1} (υ_{as}), 2944 cm^{-1} (υ_s) and bands of symmetrical and asymmetric bending vibrations of the C-H bond: 1455 cm^{-1} (δ_{as}), 1383 cm^{-1} (δ_s), and 1360 cm^{-1} (δ_s). The spectrum also contained a group of bands corresponding to C-O bending bonds in the range 1268–1046 cm^{-1} and a band of 870 cm^{-1} corresponding to C-COO bonds [33,34]. The transmission spectrum of lactide (Figure 10) contained characteristic bands: 2950 cm^{-1} from asymmetric valence vibrations of C-H, at 2924 and 3003 cm^{-1} from symmetric and asymmetric valence vibrations of C-H from CH_3. In the FTIR spectrum of lactide also appeared in bands at 1251 cm^{-1} (asymmetric valence vibrations of C-O-C in the lactonic ring), 1099 cm^{-1} (symmetric valence vibrations of C-O-C in the lactonic ring), 1769 cm^{-1} (cyclic dilactone C=O valence vibration). The bands contained a group of bands corresponding to asymmetric and symmetric bending vibration of C-H from CH_3 at 1445 and 1386 cm^{-1} and 930 cm^{-1} (COO ring breathing mode) [35]. The spectrum of lactic acid (Figure 11) contained bands mainly at 1729 cm^{-1} corresponding to the C=O stretching vibration of carboxyl groups and at 3354 cm^{-1}, corresponding to the OH stretching [36,37].

Soil samples after chloroform extraction in ultrasonic bath were subjected to FTIR analyses. The samples before (blank sample, G0) and after 20 (G20) and 24 weeks (G24) of the PLA degradation were tested. The obtained IR spectra in the Figure 12 are shown. Before degradation in extract bands at 2848 and 2916 cm^{-1} corresponding to the stretching C-H bonds, 1770–1712 cm^{-1} (characteristic for carbonyl groups) and 1462–1378 cm^{-1} (characteristic for bending vibration of C-H) were observed. Similar bands with the progress of the degradation of PLA but with lower absorbance were observed. Additionally, after 24 weeks of degradation, the characteristic band for hydroxyl groups was observed. GPC/SEC analysis also showed that the blank soil sample contained some higher molar mass compounds water and chloroform soluble. Their origin is primary and not related to degradation of PLA. On the basis of FTIR analysis, it can be concluded that the intensity of the bands decreases as a function of the degradation time (after 20 and 24 weeks).

Additionally, a band of hydroxyl groups appeared. It may come from the lactic acid, which was also detected by GC/MS.

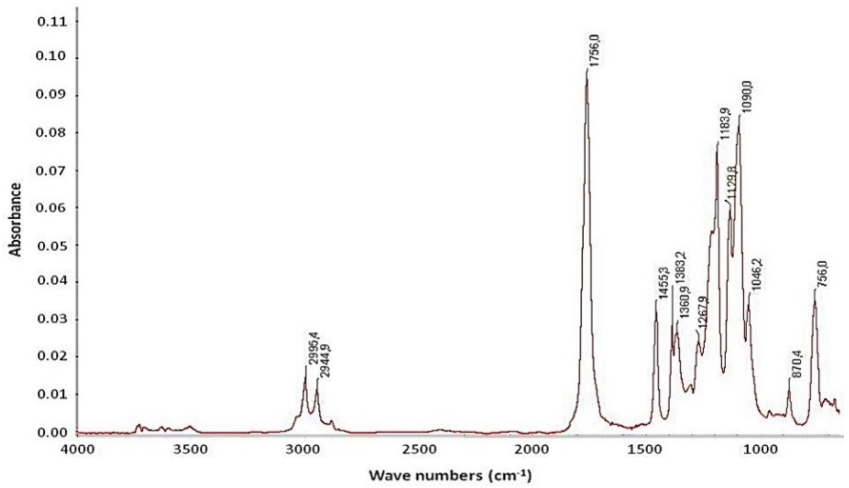


Figure 9. IR spectra for PLA stock standard solution in chloroform [concentration 3.8 g/dm^3].

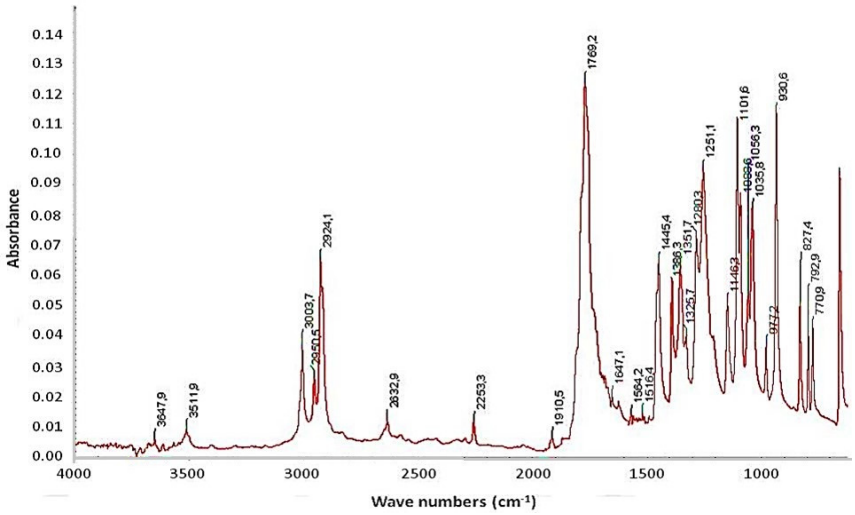


Figure 10. IR spectra for lactide stock standard solution in MeCN [concentration 1 g/dm^3].

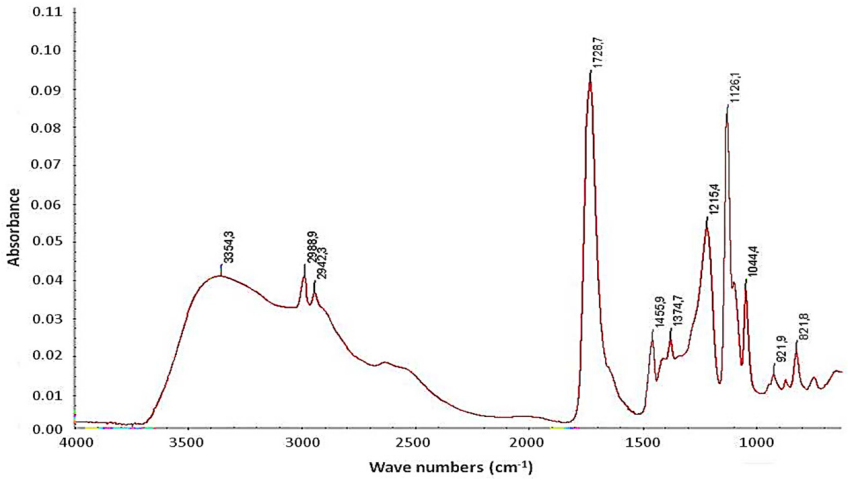


Figure 11. IR spectra for lactic acid stock standard solution in MeCN [concentration 1%].

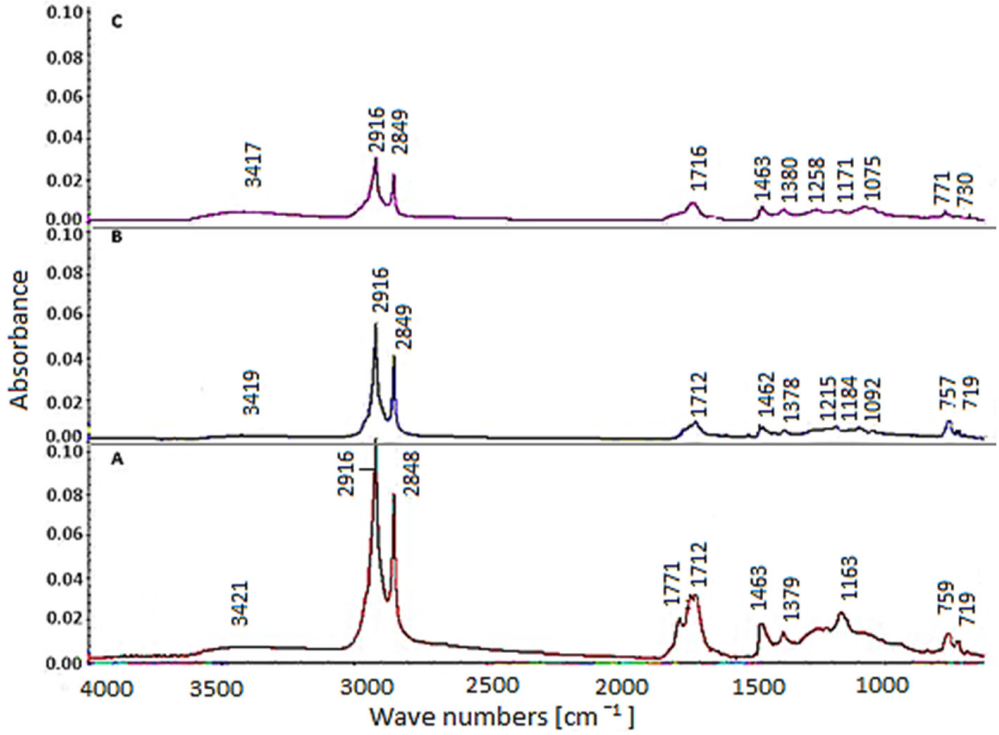


Figure 12. IR spectra for soil chloroform extracts prepared using an ultrasonic bath for (**A**) G0 sample (before degradation), (**B**) G20 sample (after 20 weeks degradation), and (**C**) G24 sample (after 24 weeks degradation).

3.2. Py-GC/MS Analysis

Analysis of PLA 6201D by Py-GC/MS technique was performed. Its degradation products based on the NIST databases were determined. Table 2 and Figure 13 presents selected degradation products of PLA.

Table 2. Selected degradation products of PLA.

Name of Degradation Product	Retention Time Range	Mass-to-Charge Ratio (*m*/*z*)
Acetaldehyde	3–5.8 min (4.874 min)	29; 43; 44
Lactic acid	5.8–9 min (6.767 min)	29; 43; 44
2-propenoic acid	9–9.4 min (9.187 min)	45; 55; 72
Pentane-2,5-dione	9.4–10.2 min (10.01 min)	29; 43; 57
Lactide	10.2–12 min (10.61 and 11.46 min)	28; 43; 45; 56
Oligomers	12–38 min	100; 128; 200; 272; 344

All tested soil samples at high temperature (400 °C) were pyrolyzed. The Py-GC/MS analysis (Figure 14) shows that the blank soil sample contained compounds (primary structures), such as lactide, oligomers. It is surprising that the obtained chromatograms suggest that the content of these compounds in the blank soil sample is significantly higher than in the samples after degradation. Due to the obtained results, other commercially available soil samples (G28, G37, and G38) by Py-GC/MS were tested (Figure 15). In these samples, different contents of these compounds were observed. One of them (G37) contained their trace amounts. However, this does not change the fact that soils contain compounds that are also degradation products of PLA. The studies performed by GC/MS technique (Figures 2–5) did not detect compounds in the blank soil sample (G0) after solvent extraction. After PLA degradation detected peaks from lactic acid and lactide in samples only after 24 weeks of process (Figures 2–5). Analysis using the pyrolytic technique provided information about the total tested sample under high-temperature conditions (400 °C), whereas GC/MS technique in which soil samples were extracted

with solvents, provided only information about water-soluble and chloroform-soluble compounds. GPC/SEC analysis in soil extracts showed the presence of compounds with higher molecular weights, undetectable by GC/MS. FTIR analysis also confirmed the presence of various compounds in extract form blank soil sample. Therefore, as a result of pyrolysis at high temperature, compounds with higher molar masses (determined in GPC/SEC and FTIR) probably degraded into low molecular mass compounds. The content of low molecular weight compounds (including lactide) and the group of oligomers in the tested samples before the biodegradation process and after 20 and 24 weeks of the process was observed. A relatively high lactide content for the blank sample compared to the other samples is observed. The results obtained for the remaining primary compounds in sample G0 also show their higher content than for G20 and G24 samples. During the PLA degradation process, a slight decrease of the oligomers content, a decrease of the lactide content, and an increase the content of smaller particles (pentane-2,5-dione) were observed.

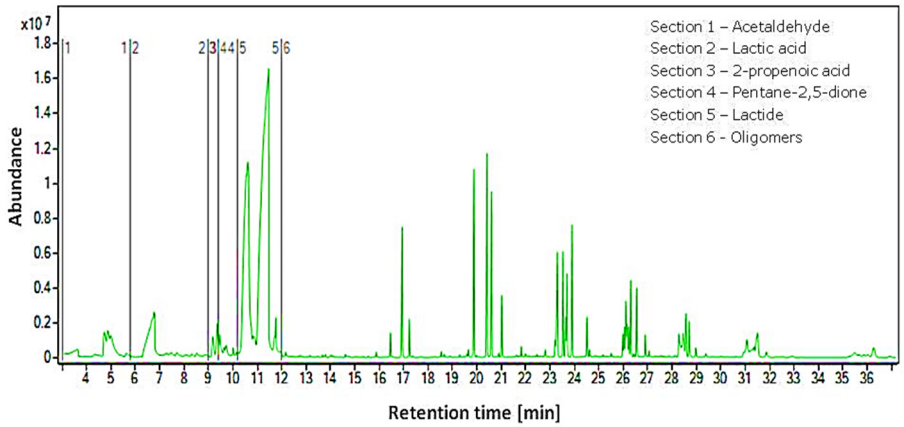


Figure 13. Py-GC/MS chromatogram obtained for the PLA sample after analysis at 400 °C using the SIM mode.

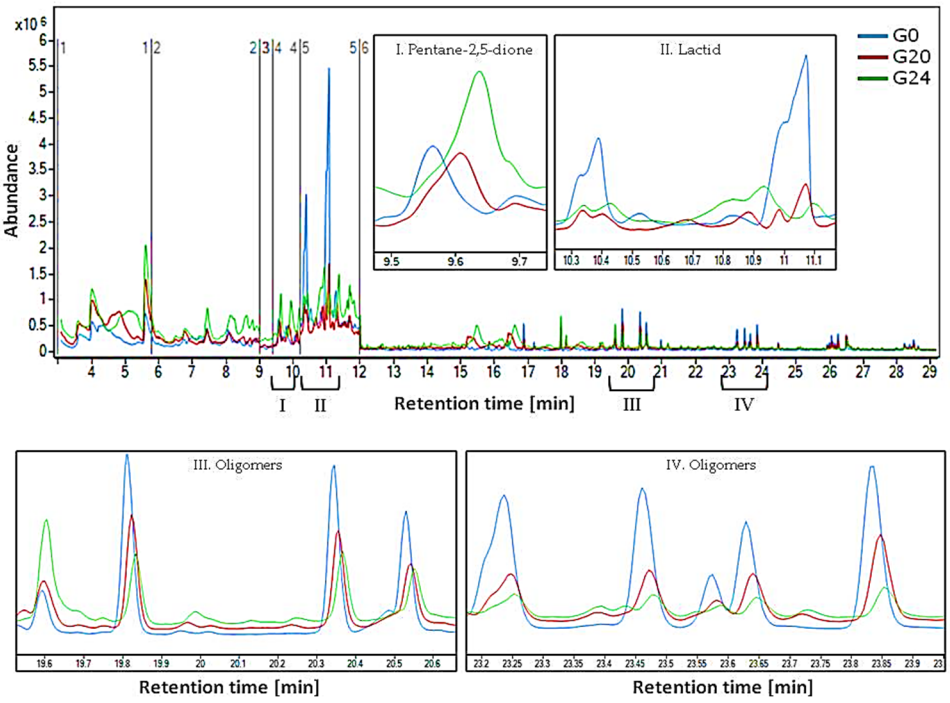


Figure 14. Chromatogram obtained for the initial soil samples and after 20 and 24 weeks of the degradation process. Analyses by Py-GC/MS technique at 400 °C using the SIM mode.

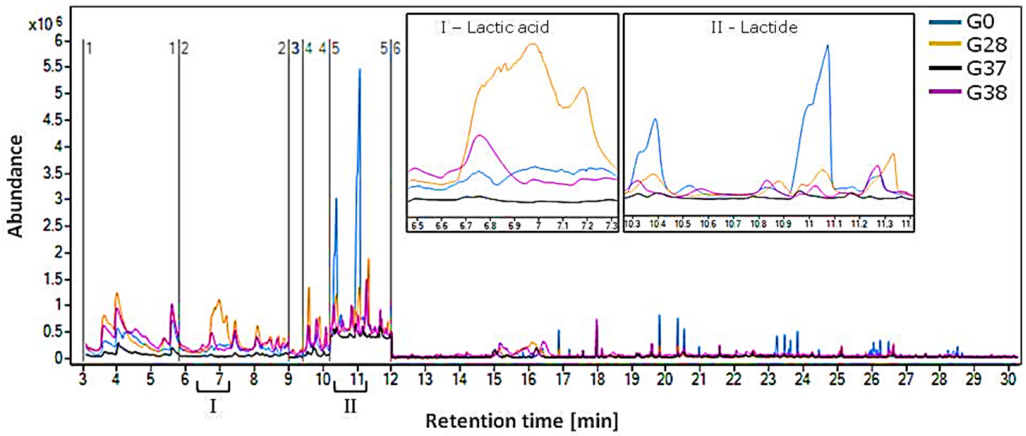


Figure 15. Chromatogram obtained for various commercial zero soils samples. Analyses by Py-GC/MS technique at 400 °C using the SIM mode.

4. Conclusions

The biodegradation process of PLA is complicated and depends on several factors, mainly the presence of water and temperature. Hydrolytic degradation is the first step in polymer degradation in soil. The penetration of water into the structure of PLA breaks the ester bonds and creates oligo- and monomeric structures. The water-soluble oligomers can then diffuse into the surrounding environment. When the rate of oligomer release is greater than the rate of water diffusion into the sample, surface erosion can be observed. Conversely, erosion is observed throughout the entire volume of the material. If the products of the hydrolytic decomposition of PLA are released very slowly from the depths of the material and at the same time increase the rate of hydrolysis, then accelerated erosion of the sample core takes place. In the second stage, water-soluble units and oligomers can be metabolized by microorganisms to simpler compounds, and finally to CO_2 and H_2O. The speed of PLA degradation is strictly dependent on environmental conditions. The higher the temperature and humidity, the faster the process will be. However, it is important to identify the most optimal conditions that will allow the metabolism of the degradation products by the microorganisms without accumulation of the oligomers in the environment [25,38]. The size and shape of the PLA itself is crucial during the biodegradation process. Larger, uniform structures decompose much slower than flat or smaller fragments. Another important factor is the conditions in the environment surrounding the polymer, such as temperature, humidity, pH, buffer capacity, and ionic strength [39].

It is assumed that for biodegradable materials over a period of 24 weeks, a loss of mass is not less than 90%. The conducted PLA biodegradation tests did not show any changes in the weight loss of the tested samples. However, erosion of samples was observed and degradation compounds penetrated into the substrate from the polymer. This may be due to various factors determining the course of the biodegradation and degradation process. Tests may indicate the chemical decomposition of the polymer with the simultaneous lack of susceptibility to decomposition caused by the action of microorganisms under given environmental conditions. The aim of the research was to prove whether products made of PLA, e.g., packaging, films, waste left in the environment, release substances that may be harmful. Therefore, soil was selected as a medium to characterize the substances potentially released from the polymer under conditions simulating the process in the environment. This is confirmed by the results of the GC/MS, Py-GC/MS, and GPC/SEC analyses, which show that the structures of short-chain oligomers, lactide and lactic acid from the polymer under conditions simulating the process in the environment were obtained. Following 24 weeks of the PLA degradation process by GC/MS technique, Soil samples after extraction in water and chloroform peaks from lactic acid and lactide in samples were observed. Using this technique, blank sample (G0-before degradation) and sample

after 20 weeks of degradation (G20) did not contain these compounds or they were in trace amount. GPC/SEC analysis detected higher molar mass compounds in the extracts from blank soil sample before degradation. However, after 24 weeks, the molar mass increased and the mass distribution changed, which may result from the degradation of PLA. The Py-GC/MS analysis showed that the blank soil sample contained compounds, which had similar structure as PLA degradation products, such as oligomers and lactide, which may come from thermal degradation of higher molar mass compounds (detected by GPC/SEC in soil extract). In soil, after 24 weeks of PLA degradation, similar compounds that were present in blank soil by the employed techniques were determined. It should be emphasized that the oligomer structures at the end of the experiment (after 24 weeks) are still present in the soil, which proves that only the hydrolytic degradation of the polymer took place. However, a surprising phenomenon was the obtaining of structures similar to the degradation products of PLA in soil samples not subjected to any experiments (blank samples). The presence of low molecular mass compounds, such as lactic acid or lactide as well as oligomeric structures proves the richness of the soil as a research matrix. The occurrence of these compounds in soil may be of primary character, not related to polymer degradation. Their occurrence was confirmed by three applied research techniques (GC/MS, Py-GC/MS, and GPC/SEC). This phenomenon was confirmed by the obtained research results for various commercially available soils. After Py-GC/MS analysis of the blank soil, structures were similar as in the soil after PLA degradation. Therefore, we can conclude that the release of PLA compounds should not constitute new environmental pollution.

The obtained research reflects new light into the problem of the presence of plastics in the environment and deliver extremely important information and new approaches to further soil analysis.

Among the available literature, there are no reports on the residues of PLA degradation products in soil and their possible impact on the environment. The issue of possible accumulation of compounds from the decomposition of PLA in the environment and the answer to the question as to what extent this polymer is a good alternative to plastics seems to be crucial.

Author Contributions: Conceptualization, M.K.-W.; formal analysis, M.K.-W.; investigation, M.K.-W., K.G.-J., A.M. and J.J.-P.; methodology, M.K.-W., K.G.-J. and A.M.; supervision, M.K.-W.; writing—original draft, M.K.-W., K.G.-J.; writing—review and editing, A.M., J.J-P. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by Łukasiewicz-Institute of Biopolymers and Chemical Fibres within the statutory activity in 2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. European Parliament. Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment (Text with EEA relevance). *Off. J. Eur. Union* **2019**, *155*, 1–9.
2. Behuria, P. *The Comparative Political Economy of Plastic Bag Bans in East Africa: Why Implementation Has Varied in Rwanda, Kenya and Uganda*; GDI Working Paper; The University of Manchester: Manchester, UK, 2019; Volume 37, ISBN 978-1-909336-72-8.
3. UN Environment Programme. Available online: https://www.unep.org/news-and-stories/story/kenya-bans-single-use-plastics-protected-areas (accessed on 1 January 2021).
4. Raschip, I.E.; Fifere, N.; Varganici, C.-D.; Dinu, M.V. Development of antioxidant and antimicrobial xanthan-based cryogels with tuned porous morphology and controlled swelling features. *Int. J. Biol. Macromol.* **2020**, *156*, 608. [CrossRef]
5. Wu, F.; Misra, M.; Mohanty, A.k. Challenges and new opportunities on barrier performance of biodegradable polymers for sustainable packaging. *Prog. Polym. Sci.* **2021**, *117*, 101395. [CrossRef]

6. Pinato, L.; Bonifacio, M.A.; De Giglio, E.; Santovito, E.; Cometa, S.; Bevilacqua, A.; Baruzzi, F. Biopolymer hybrid materials: Development, characterization, and food packaging applications. *Food Packag. Shelf Life* **2021**, *28*, 100676. [CrossRef]
7. Liu, Y.; Ahmed, S.; Sameen, D.E.; Wang, Y.; Lu, R.; Dai, J.; Li, S.; Qin, W. A review of cellulose and its derivatives in biopolymer-based for food packaging application. *Trends Food Sci. Technol.* **2021**, *112*, 532. [CrossRef]
8. Rajeshkumar, G.; Seshadri, S.A.; Devnani, G.L.; Sanjay, M.R.; Siengchin, S.; Maran, J.P.; Anuf, A.R. Environment friendly, renewable and sustainable poly lactic acid (PLA) based natural fiber reinforced composites—A comprehensive review. *J. Clean. Prod.* **2021**, *310*, 127483. [CrossRef]
9. Shankara, S.; Wangb, L.-F.; Rhima, J.W. Incorporation of zinc oxide nanoparticles improved the mechanical, water vapor barrier, UV-light barrier, and antibacterial properties of PLA-based nanocomposite films. *Mater. Sci. Eng. C* **2018**, *93*, 289. [CrossRef]
10. Liu, Y.F.; Jiang, S.Y.; Yan, W.; He, M.; Qin, J.; Qin, S.H.; Yu, J. Crystallization Morphology Regulation on Enhancing Heat Resistance of Polylactic Acid. *Polymers* **2020**, *12*, 1563. [CrossRef]
11. Delgado-Aguilar, M.; Puig, R.; Sazdovski, I.; Fullana-I-Palmer, P. Polylactic Acid/Polycaprolactone Blends: On the Path to Circular Economy, Substituting Single-Use Commodity Plastic Products. *Materials* **2020**, *13*, 2655. [CrossRef]
12. Vanaei, H.; Shirinbayan, M.; Deligant, M.; Raissi, K.; Fitoussi, J.; Khelladi, S.; Tcharkhtchi, A. Influence of process parameters on thermal and mechanical properties of polylactic acid fabricated by fused filament fabrication. *Polym. Eng. Sci.* **2020**, *60*, 8. [CrossRef]
13. Marczak, D.; Lejcuś, K.; Misiewicz, J. Characteristics of biodegradable textiles used in environmental engineering: A comprehensive review. *J. Clean. Prod.* **2020**, *268*, 20. [CrossRef]
14. Rocha, D.B.; Souza de Carvalho, J.; Aparecida de Oliveira, S.; Santos Rosa, D. A new approach for flexible PBAT/PLA/CaCO3 films into agriculture. *J. Appl. Polym. Sci.* **2018**, *135*, 46660. [CrossRef]
15. Lunt, J.; Shafer, A.L. Polylactic Acid Polymers from Com. Applications in the Textiles Industry. *J. Ind. Text.* **2000**, *29*, 3, 191. [CrossRef]
16. Zaaba, N.F.; Jaafar, M. A review on degradation mechanisms of polylactic acid: Hydrolytic, photodegradative, microbial, and enzymatic degradation. *Polym. Eng. Sci.* **2020**, *60*, 2061. [CrossRef]
17. Żenkiewicz, M.; Richert, J. Synteza, właściwości i zastosowanie polilaktydu. *Przetwórstwo Tworzyw* **2009**, *5*, 192. (In Polish)
18. Inkinen, S.; Hakkarinen, M.; Albertsson, A.-C.; Södergård, A. From lactic acid to poly(lactic acid) (PLA): Characterization and analysis of PLA and its precursors. *Biomacromolecules* **2011**, *12*, 3, 523. [CrossRef]
19. Tokiwa, Y.; Calabia, B.P. Biodegradability and biodegradation of poly(lactide). *Appl. Microbiol. Biotechnol.* **2006**, *72*, 2, 244. [CrossRef]
20. Tokiwa, Y.; Jarerat, A. Biodegradation of poly(l-lactide). *Biotechnol. Lett.* **2004**, *26*, 771. [CrossRef]
21. Khabbaz, F.; Karlsson, S.; Albertsson, A.-C. PY-GC/MS an effective technique to characterizing of degradation mechanism of poly (L-lactide) in the different environment. *J. Appl. Polym. Sci.* **2000**, *78*, 2369. [CrossRef]
22. Ingrao, C.; Tricase, C.; Cholewa-Wójcik, A.; Kawecka, A.; Rana, R.; Siracusa, V. Polylactic acid trays for fresh-food packaging: A Carbon Footprint assessment. *Sci. Total Environ.* **2015**, *537*, 385. [CrossRef]
23. Alaerts, L.; Augustinus, M.; Van Acker, K. Impact of Bio-Based Plastics on Current Recycling of Plastics. *Sustainability* **2018**, *10*, 1487. [CrossRef]
24. Maqsood, M.; Langensiepen, F.; Seide, G. Investigation of melt spinnability of plasticized polylactic acid biocomposites-containing intumescent flame retardant. *J. Therm. Anal. Calorim.* **2020**, *139*, 305. [CrossRef]
25. Nowak, B.; Pająk, J. Biodegradacja polilaktydu (PLA). *Arch. Waste Manag. Environ. Prot.* **2010**, *12*, 2. (In Polish)
26. Karamanlioglu, M.; Robson, G.D. The influence of biotic and abiotic factors on the rate of degradation of poly(lactic) acid (PLA) coupons buried in compost and soil. *Polym. Degrad. Stab.* **2013**, *98*, 2063–2071. [CrossRef]
27. Ehlers, B.K.; Berg, M.P.; Staudt, M.; Holmstrup, M.; Glasius, M.; Ellers, J.; Tomiolo, S.; Madsen, R.M.; Slotsbo, S.; Penuelas, J. Plant Secondary Compounds in Soil and Their Role in Belowground Species Interactions. *Trends Ecol. Evol.* **2020**, *35*, 8, 716. [CrossRef]
28. Laudicina, V.A.; Palazzolo, E.; Badalucco, L. Natural Organic Compounds in Soil Solution: Potential Role as Soil Quality Indicators. *Curr. Org. Chem.* **2013**, *17*, 2991. [CrossRef]
29. Food and Agriculture Organization of the United Nations (FAO) and the United Nations Environment Programme (UNEP). *Global Assessment of Soil Pollution—Summary for Policy Makers*; FAO and UNEP: Rome, Italy, 2021; ISBN 978-92-5-134448-4.
30. Silva, D.D.; Kaduri, M.; Poley, M.; Adir, O.; Krinsky, N.; Shainsky-Roitman, J.; Schroeder, A. Biocompatibility, biodegradation and excretion of polylactic acid (PLA) in medical implants and theranostic systems. *Chem. Eng. J.* **2018**, *340*, 9. [CrossRef]
31. Visan, A.I.; Popescu-Pelin, G.; Socol, G. Degradation Behavior of Polymers Used as Coating Materials for Drug Delivery—A Basic Review. *Polymers* **2021**, *13*, 1272. [CrossRef]
32. CSN EN 14995:2009 Plastics—Evaluation of Compostability—Test Scheme and Specifications. Available online: https://www.en-standard.eu/csn-en-14995-plastics-evaluation-of-compostability-test-scheme-and-specifications/ (accessed on 7 December 2021).
33. Silverstein, R.M.; Webster, F.X.; Kieml, D.J. *Spectrometric Identification of Organic Compounds*, 7th ed.; John Wiley & Sons: New York, NY, USA, 2005; ISBN 0-471-39362-2.
34. Miros-Kudra, P.; Gzyra-Jagieła, K.; Kudra, M. Physicochemical Assessment of the Biodegradability of Agricultural Nonwovens Made of PLA. *Fibres Text. East. Eur.* **2021**, *1*, 26. [CrossRef]
35. Nikolic, L.; Ristic, I.; Adnadjevic, B.; Nikolic, V.; Jovanovic, J.; Stankovic, M. Novel Microwave-Assisted Synthesis of Poly(D,L-lactide): The Influence of Monomer/Initiator Molar Ratio on the Product Properties. *Sensors* **2010**, *10*, 5063. [CrossRef]

36. Tang, W.; Wang, B.; Li, J.; Li, Y.; Zhang, Y.; Quan, H.; Huang, Z. Facile pyrolysis synthesis of ionic liquid capped carbon dots and subsequent application as the water-based lubricant additives. *J. Mater. Sci.* **2019**, *54*, 1171. [CrossRef]
37. Devi, P.N.; Sathiyabama, J.; Rajendran, S. Study of surface morphology and inhibition efficiency of mild steel in simulated concrete pore solution by lactic acid–Zn2+ system. *Int. J. Corros. Scale Inhib.* **2017**, *6*, 18. [CrossRef]
38. Jarerat, A.; Tokiwa, Y. Poly(L-lactide) degradation by *Saccharothrix waywayandensis*. *Biotechnol. Lett.* **2003**, *25*, 401. [CrossRef]
39. Artham, T.; Doble, M. Biodegradation of aliphatic and aromatic polycarbonates. *Macromol. Biosci.* **2008**, *8*, 14–24. [CrossRef] [PubMed]