Phyto-remediation of Land Polluted with Non-Recyclable Plastic

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Abstract

The ability of soybean (*Glycine max*) and vetch plants (*Vicia*) to stimulate the degradation of low-density polyethylene (LDPE) in soil was tested in the vegetative experiments under laboratory conditions. It was shown that the presence of LDPE strips in soil had no toxic effects on either of the plants, and that, in the case of soybeans (*Glycine max*), it stimulated plant growth. It has been shown that soybeans (*Glycine max*) and vetch (*Vicia*) can stimulate the degradation of LDPE depending on the particularities of the soil and the presence of plant growth stimulating factors. The vetch plants (*Vicia*) were relatively more effective in stimulating the LDPE degradation in case of unpolluted soil, collected from the forest. Soybean plants (*Glycine max*) were relatively more efficient under polluted soil conditions and lead to the highest rate of LDPE degradation (2.3% in 39 days) – in the variant with seed bacterization by *Rhizobium japonicum* RB-06. The ability to ensure a significant degradation of LDPE, under the real toxic conditions of polluted soil, highlighted the perspectivity of soybean plants (*Glycine max*) for the development of phyto-remediation procedures of lands polluted by non-recyclable plastic and other contaminants

Keywords: LDPE, non-recyclable plastics, rhizobia, soil pollution, soil phyto-remediation, soybeans (*Glycine max*).

1. Introduction

In most countries, the problem of environmental pollution with non-recyclable plastic products persists, including low-density polyethylene (LDPE). Every year, more than 100-300 thousand tons of plastic waste are produced in our country, most of which are tossed into the environment (including about 1050 landfills) without any processing [1,2].

Once in the soil, plastic materials break down under the action of biotic and abiotic factors into smaller particles, which exert a negative impact on soil health, plant development and their productivity [3-8]. Microplastic from soil and water enters the plants through the cracks via the lateral roots [9]. Plastic buildup in plants has detrimental effects on the yield and nutritional value of produced goods, and, as a result, endangers food security and the sustainable development of agriculture [4,6,7,9]. The creation of corrective measures is necessary due to the detrimental effects of plastic soil pollution.

The development of biological degradation processes, including the use of phyto-remediation, has recently received attention [10-13] due to the absence of effective chemical and physical methods for the removal of plastic and other hazardous pollutants from the soil. Phyto-remediation is the process of removing, detoxifying, and destroying hazardous chemicals from the environment using plants, plant-associated microorganisms from the rhizosphere, soil amendments, and agronomic methods. Phyto-remediation is already being successfully used in

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the practice of purifying agricultural land and groundwater, urban, agricultural, and industrial [14] and is increasingly being studied as a method of restoring soils polluted with various organic and inorganic substances (persistent organic pollutants, heavy metals, etc.) [9].

However, at this time, very little is known about the potential applications of phyto-remediation for soils contaminated with plastic pollutants.

In additional studies, it was shown that soybean (*Glycine max*) and leguminous vetch plants (*Vicia*) bacterized with particular rhizobia strains and grown in typical weak humiferous chernozem had an increased resistance to the toxic action of LDPE introduced into the soil at concentrations up to 5 g/kg.

Additionally, in some instances, the presence of LDPE dramatically promoted their growth.

Based on the foregoing, it was determined in this study whether soybean (*Glycine max*) and vetch plants (*Vicia*) may encourage the breakdown of LDPE in the soil.

2. Materials and methods

In a series of experimental vegetation tests, the capacity of soybean (*Glycine max*) and vetch plants (*Vicia*) to promote LDPE decomposition in soil was examined.

Unpolluted, virgin forest soil and soil contaminated with plastic debris (including LDPE) and other contaminants were the two contrasted plots from which soil samples were chosen, standardized, and classified.

The conditions of severe ecological stress in the soil of the polluted field were highlighted by the comparative examination of the features of the chosen soils (Table 1).

Unlike the version of the unpolluted soil, the microbial biomass was 27 times lower, and the metabolic coefficient (the indicator of the state of ecological stress for soil microorganisms) – 99 times higher.

In a climate chamber with consideration for the parameters of illumination, humidity, ventilation, and temperature, three iterations of vegetative studies using pots with soil (300 g/pot) were conducted. Soybean (*Glycine max*) and vetch plants (*Vicia*) were grown to the budding-flowering stage.

In total, the following variants were provided:

(1) Control – soil sown with non-bacterized seeds;

(2) RZ - soil sown with soybeans (*Glycine max*) or vetch (*Vicia*) bacterized respectively with *Rhizobium janopicum* RB-06 and *Rhizobium leguminosarum* RB-02;

(3) LDPE – soil with LDPE introduced in the form of 2 strips cut longitudinally and 2 strips cut transversely for each variant-repetition, sown with non-bacterized seeds;

(4) RZ-LDPE – soil with LDPE sown with bacterized seeds.

The degree of germination, the length of the roots, the height of the plants (aerial parts), the dry mass of the plants (together with the roots), the number and the dry mass of the root nodules were determined (where possible) at the conclusion of the vegetative experiments in each variant, as well as the degree of LDPE degradation (decrease in mass at the conclusion of the experiment).

Table 1. Soil properties from vegetation experiments

Land	CSO[1] [%]	pH	W[2] [%]	BMS[3] [μg C/g]	RS[4] [μg C-CO_2/g/hour]	*qCO_2*[5] [C-CO_2/mg biomass/hour]
Uncontaminated soil	6.71±0.02	6.9	28.1	914.2±22.4	0.39±0.03	0.42±0.03
Contaminated soil	4.88±0.03	7.9	19.7	33.8±8.3	1.42±0.09	41.96±3.05

[1] CSO - organic matter content.

[2] W - soil moisture at 40% of water holding capacity.

[3] BMS - soil microbial biomass.

[4] RS - soil respiration.

[5] qCO_2 - metabolic coefficient.

Statistical analysis is shown using confidence interval, P=0.95.

3. Results and discussion

Most variations of the two examined soils did not significantly influence plant development or the formation of nodules on their roots as a result of the introduction of LDPE (Table 2 and 3).

Only the variant with bacterized soybeans (*Glycine max*) in polluted soil and the second variant with vetch (*Vicia*) without bacterization showed significant changes. In the polluted soil variant, the number of root nodules was reduced to zero, while the root length increased by 56.4% compared to the absolute control in the variant with bacterized soybeans (*Glycine max*).

These modifications demonstrated that soybeans (*Glycine max*) can also be promoted in the presence of a specific pollutant and that vetch plants (*Vicia*) are quite resistant to the damaging effects of LDPE.

Out of 6 examined characteristics, consistent and significantly negative changes were only observed in terms of plant height (which reduced on average by 59.2%), indicating that soybean (*Glycine max*) plants, in contrast to vetch (*Vicia*), were much more resilient to the unfavorable conditions of the polluted soil. For comparison, it was found that the vetch plants (*Vicia*) significantly deteriorated under the conditions of the polluted soil, with average decreases in root length, height, and dry weight of the plants of 2.7, 1.5, and 1.7 times, respectively, and average decreases in the number of root nodules of 21 to 55, and in one case, as was already mentioned, of zero.

Table 2. The influence of LDPE on soybean (*Glycine max*) plant growth and nodulation processes on roots under polluted and unpolluted soil conditions with non-recyclable plastic and other contaminants

Variant	Seed germination [%]	Root length [cm]	Plant height [cm]	Dry mass of plants [g]	No of nodules	Dry mass of nodules [mg]
			Forest soil(unpolluted)			
Control[1]	66.67±24.66	19.70±3.75	85.72±6.91	3.97±0.38	-	-
RZ[2]	80.93±24.72	22.37±4.92	96.83±16.19	5.45±2.25	23.67±7.95	16.37±5.07
LDPE[3]	66.67±24.66	18.96±4.24	80.56±14.70	4.21±1.17	-	-
RZ+LDPE[4]	71.40±16.18	19.37±1.53	95.89±13.43	4.95±1.12	14.67±6.91	8.70±3.43
			Polluted soil			
Control[1]	71.43±27.99	15.66±1.39	35.78±4.52	3.10±0.53	-	-
RZ[2]	90.47±18.68	19.82±1.57	39.90±4.42	3.75±0.33	9.33±2.85	18.03±3.37
LDPE[3]	66.67±24.66	21.81±1.32	36.49±5.87	3.25±0.28	-	-

[1] Control - soil (without LDPE) sown with non-sterilized soybeans (*Glycine max*).
[2] RZ - soil with *Rhizobium japonicum* RB-06 strain.
[3] LDPE - soil treated with LDPE and subsequently sown with non-sterilized seeds.
[4] RZ+LDPE - soil treated with LDPE and subsequently sown with bacterized seeds.
Statistics are shown by confidence interval at P=0.95.

Table 3. The influence of LDPE on the growth processes of vetch plants (*Vicia*) and the formation of nodules on their roots in soil conditions polluted and unpolluted with non-recyclable plastic and other contaminants

Variant	Root length [cm]	Plant height [cm]	Dry mass of plants [g]	No of nodules
Forest soil (unpolluted)				
Control[1]	33.67±6.53	81.83±6.33	0.32±0.06	14.50±5.67
RZ[2]	24.33±6.91	73.00±1.60	0.22±0.03	9.00±2.95
LDPE[3]	32.33±6.91	74.00±5.86	0.23±0.02	8.00±3.20
RZ+LDPE[4]	31.83±5.48	78.67±4.77	0.37±0.12	9.50±2.41
Polluted soil				
Control[1]	11.75±2.02	50.83±5.17	0.14±0.02	0.67±0.65
RZ[2]	9.50±1.66	48.83±8.87	0.15±0.03	0.17±0.33
LDPE[3]	10.00±1.34	45.50±6.00	0.13±0.03	00.00±0.00
RZ+LDPE[4]	15.33±2.51	62.17±7.25	0.19±0.11	1.67±1.49

[1] Control - soil (without LDPE) sown with non-sterilized seeds s.
[2] RZ - soil sown with seeds sterilized with *Rhizobium leguminosarum* RB-02 strain.
[3] LDPE - soil treated with LDPE and subsequently sown with non-sterilized seeds.
[4] RZ+LDPE - soil treated with LDPE and subsequently sown with bacterized seeds.
Statistics are shown by confidence interval at P=0.95.

The measurement of LDPE degradation level revealed, to varying degrees, the phyto-remedial potential of both plants. The reduction in LDPE mass, which varied from 0.4% to 2.3% depending on the soil and plant, occurred in all studied versions, according to the results (Table 4 and 5).

The polluted soil where the sterilized soybeans (*Glycine max*) were cultivated, showed the largest drop in LDPE mass (-2.9 mg). The LDPE drop was 3.5-6.5 times lower in the other soybean (*Glycine max*) varieties, even those grown on virgin forest soil, where it was much greater.

Under unpolluted forest soil circumstances, vetch plants (*Vicia*) were relatively more successful at promoting LDPE degradation; the variety with the best decrees rate of LDPE was 4.2 times higher than that of the best soybean (*Glycine max*) variant.

Table 4. LDPE degradation in tests experiments with soybean (*Glycine max*) vegetation

Variant	Initial mass of applied LDPE tapes [g]	Reducing the mass of LDPE tapes [mg]	Degree of degradation of LDPE [%]
Forest soil (duration of experiment - 32 days)			
LDPE[1]	0.254±0.003	0.450±0.148	0.353±0.114
RZ+LDPE[2]	0.256±0.009	0.608±0.318	0.476±0.246
Polluted soil(duration of experiment - 39 days)			
LDPE[1]	0.256±0.008	1.208±0.865	0.934±0.665
RZ+LDPE[2]	0.256±0.006	2.917±0.757	2.261±0.551

[1] LDPE - soil treated with LDPE and subsequently sown with unsterilized seeds.
[2] RZ+LDPE - soil treated with LDPE and subsequently sown with seeds bactericized with Rhizobium japonicum RB-06 strian. Statistics are shown by confidence interval at P=0.95.

Table 5. Degradation of LDPE in vetch (*Vicia*) vegetation experiments

Variant	Initial mass of applied LDPE tapes [g]	Reducing the mass of LDPE tapes [mg]	Degree of degradation LDPE [%]
Forest soil (duration of experiment - 32 days)			
LDPE[1]	0.257±0.009	2.550±1.489	1.953±1.110
RZ+LDPE[2]	0.256±0.007	0.558±0.382	0.432±0.290
Polluted soil (duration of experiment –39 days)			
LDPE[1]	0.265±0.006	0.975±0.666	0.736±0.494
RZ+LDPE[2]	0.257±0.001	1.558±0.352	1.210±0.276

[1] LDPE - soil treated with LDPE and subsequently sown with non-sterilized seeds

[2] RZ+LDPE - soil treated with LDPE and subsequently sown with seeds bacterised with the *Rhizobium japonicum* RB-06 strain.

Statistics are shown by confidence interval at P=0.95.

Leguminous plants may be used to encourage the biodegradation of LDPE in the soil due to LDPE deterioration in the examined forms. By choosing plants suited for particular soil conditions and encouraging their growth, it may be possible to increase the biodegradation of LDPE in soil. This is demonstrated by the 6.4 times larger difference between the highest and lowest degree of degradation of LDPE from different variants. A key advantage of this plant in relation to the development of phyto-remediation techniques for lands polluted with non-recyclable plastic and other toxins is implied by the capacity of soy to assure the significant decomposition of LDPE, specifically in the truly toxic conditions of the polluted soil.

However, the comparatively low values of the degree of LDPE mass reduction seen in trials show that, from a practical standpoint, the rates of LDPE biodegradation are still fairly moderate. In the best case scenario, 1725 days would have been required for full LDPE degradation. Finding new strategies to accelerate LDPE biodegradation is obviously beneficial for the effectiveness of phyto-remediation operations on LDPE-polluted fields.

4. Conclusions

Leguminous plants can aid in the phyto-remediation of land that has been contaminated by non-recyclable plastic trash and can encourage the biodegradation of LDPE in the soil.

The characteristics of the soil, the phyto-remedial plant, and the presence of elements promoting this plant's growth and development under polluted soil conditions determine the rates of LDPE biodegradation.

In the true toxic conditions of soil contaminated with non-recyclable plastic and other toxins, soybean (*Glycine max*) plants can significantly degrade LDPE.

The search for new strategies to accelerate the growth of phyto-remedial plants and the rates of non-recyclable plastic biodegradation is encouraged in order to develop viable methods for phyto-remediation of polluted soil.

References

1. Afzal. M., Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. In: An approachtolow-density polyethylene biodegradation by *Bacillus amyloliquefaciens*. Das. M.P., Kumar, S. Biotech. 2015. 5. 81-86
2. Kasirajan. S., Ngouajio. M., Polyethylene and biodegradable mulches for agricultural applications: a review. Agron. Sustain. Dev. 2012. 32. 501
3. Lianzhen. L., et al., Effective uptake of submicrometre plastics by crop plants via a crack-entry mode. Nature Sustainability. 2020. 3. 929-937
4. Tribedi. P., Sil. A.K., Low-density polyethylene degradation by *Pseudomonas* sp.AKS2 biofilm. Environmental Science and Pollution Research. 2013. 20(6). 4146-4153
5. Colette. W., Wallace. J.N., Editorial: Plastic Pollution: An Ocean Emergency. Marine Turtle Newsletter
6. Derraik Jose, G.B., The pollution of the marine environment by plastic debris: a review. Marine Pollution Bulletin. 2002. 44(9). 842-852
7. Hopewell. J., Dvorak. R., Kosior. E., Plastics recycling: Challenges and opportunities. Philosophical Transactions of the Royal Society B: Biological Sciences. 2009. 364(1526). 2115-2126

8. Nizzetto. L., Langaas. S., Futter. M., Pollution: Do microplastics spill on to farm soils? Nature, 2016, 537. 488
9. Pilon-Smits. E., Phytoremediation. Annual Review of Plant Biology. 2005. 56. 15-39
10. Shalini. R., Sasikumar. C., Biodegradation of low-density polythene materials using microbial consortium - an overview. International Journal of Pharmaceutical and Chemical Sciences. 2015. 4(4). 507-514
11. Souza. M., et al., Microplastics as an emerging threat to terrestrial ecosystems. Global Change Biology
12. Weithmann. N., et al., Organic fertilizer as a vehicle for the entry of microplastic intro the environment. Science Advances. 2018. 4(4). doi:10.1126/sciadv.aap8060
13. Xiao-Dong. S., et al., Differentially charged nanopastics demonstrate distinct accumulation in *Arabidopsis thaliana*. Nature Nanotechnology. 2020. 755-760
14. https://www.activestudy.info/simbiotic-azotfik