engineering proceedings

MDPI

Proceeding Paper

Some Microbiological Characteristics of the Biofilm on the Surface of Pre-Production Pellets of Polypropylene Microplastics after Short Exposure in the Soil †

Nataliia Tkachuk [1,*] and Liubov Zelena [2]

[1] Department of Biology, T.H. Shevchenko National University "Chernihiv Colehium", Chernihiv, Ukraine
[2] Department of Biotechnology, Leather and Fur of the Kyiv National University of Technologies and Design, Kyiv, Ukraine; zelenalyubov@gmail.com
* Correspondence: nataliia.smykun@gmail.com; Tel.: +380661730260
† Presented at the 4th International Electronic Conference on Applied Sciences, 27 October–10 November 2023; Available online: https://asec2023.sciforum.net/.

Abstract: The aim of this study was to investigate some chemical and microbiological characteristics of the soil and the surface biofilm of both polypropylene microplastics and quartz sand. The exposure of sterile samples lasted for 30 days in the soil of a residential area. Some initial chemical and microbiological characteristics of the soil were studied, as well as microbiological characteristics of the biofilm on the surface of materials. This expands the understanding of biofilm formation processes on the surface of microplastics in soil and can be used in processes for removing harmful materials from soil.

Keywords: biofilm; chemical characteristics; polypropylene microplastics; soil; sulfate-reducing bacteria; the total microbial count

Citation: Tkachuk, N.; Zelena, L. Some Microbiological Characteristics of the Biofilm on the Surface of Pre-Production Pellets of Polypropylene Microplastics after Short Exposure in the Soil. *Eng. Proc.* **2023**, *52*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: date

1. Introduction

Microplastics are defined as synthetic organic polymer particles with a size (or, more precisely, the largest size) <5 mm [1]. The problem of environmental pollution caused by microplastic accumulation in various ecosystems draws attention in the whole world [1–4] and in Ukraine specifically [5,6].

Biological activity of microorganisms on polymeric materials that get into the soil, in particular, as garbage, leads to biofilm formation and initialization of material biodegradation occurs [7,8]. Among soil microorganisms, as biodegraders of synthetic polymers, heterotrophic bacteria and sulfate-reducing bacteria (SRB) deserve attention, since they are part of the plastisphere and are able to form a biofilm on the surfaces of plastics [8–12]. The aim of this study was to investigate some chemical and microbiological characteristics of the soil and the surface biofilm of both polypropylene microplastic and quartz sand.

2. Materials and Methods

Particles of quartz sand (Poland) and primary pellets of polypropylene microplastic (Republic of Serbia) with a size of 3–5 mm in the amount of 15 pieces for each of the three repetitions of each variant were used for the research. The mass of 15 samples in each case was determined by weighing in order to determine the volume of materials used in the study. Before the start of the experiment, the biofilm was removed from the samples by ultrasound [13], the samples were sterilized with 70% ethyl alcohol, which was removed by washing the samples in sterile distilled water before applying them to the soil. The samples were mixed with the soil in sterile polypropylene containers, each of 120 mL and

with 5 holes of 1 mm diameter at the bottom. Open containers were placed in the soil at a depth of 20-25 cm.

The exposure of samples lasted for 30 days in the sod-podzolic soil of a residential area (which was not subjected to anthropogenic influence) in Chernihiv city, Ukraine (51°29′58″ N, 31°16′08″ E). Some initial chemical characteristics of the soil (nitrate nitrogen, ammonium nitrogen, pH, sulfates, chlorides, sulfur, humidity) were studied in the laboratory of Government Agency "Chernihiv Regional Center for Disease Control and Prevention" of Ministry of Health of Ukraine (Chernihiv, Ukraine) by the methods recommended by regulatory documents of Ukraine. A soil sample selected by a generally accepted method was used for the study [14]. Microbiological characteristics of the soil and the biofilm on the surface materials were studied in the laboratory of T.H. Shevchenko National University "Chernihiv Colehium" (Chernihiv, Ukraine): the total microbial count (TMC)—deep inoculation of soil suspension dilutions (or dilutions of physiological solution of NaCl with biofilm removed by ultrasound) in meat-peptone agar, aerobic cultivation conditions at 37 °C; the number of sulfate-reducing bacteria—inoculation of dilutions of soil suspension (or dilutions of physiological solution of NaCl with biofilm removed by ultrasound) into liquid Postgate's "C" medium, anaerobic cultivation conditions at 29 ± 2 °C. The preparation of the soil suspension and its dilutions was carried out according to the generally accepted method [15]. Samples of materials were taken from the soil, washed with a sterile physiological solution of NaCl from the cells of microorganisms that did not attach to the surface, and then the biofilm was removed from the surface to determine the number of the indicated groups of microorganisms. The biofilm of the surface of quartz sand particles and polypropylene microplastics (pre-production pellets) was removed in a physiological NaCl solution by ultrasound (28 kHz) as described earlier [16].

When analyzing the results, statistical methods were used: the number of bacteria in the liquid medium was determined using McCready tables; the number of bacteria on a dense medium was determined by calculating the standard error of the arithmetic mean.

3. Results and Discussion

The values of investigated chemical characteristics are within the limits of normative characteristics (Table 1).

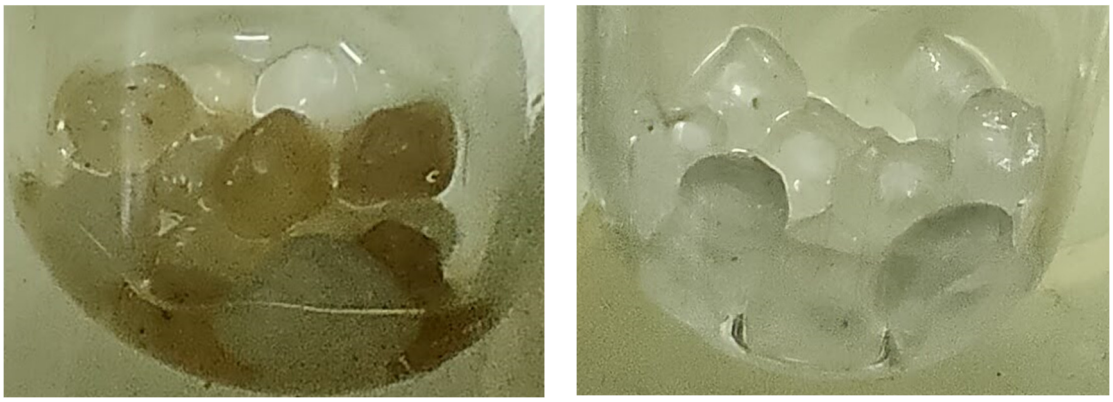
Table 1. Chemical characteristics of the studied soil.

Characteristic	Result	Limit Permissible Concentration
Nitrate nitrogen	<4.0 mg/kg	130.0 mg/kg
Ammonium nitrogen	<1.0 mg/kg	Not normalized
pH	7.05 units pH	Not normalized
Sulfates	9.4 mg/kg	Not normalized
Chlorides	500.0 mmol/100 g	Not normalized
Sulfur	<2.0 mg/kg	160.0 mg/kg
Humidity	4.3%	Not normalized

It was established that the studied soil is uncontaminated in terms of sanitary-hygienic and microbiological safety—TMC was 1.26 ± 0,15 × 10^5 colony forming units (CFU)/1 g abs. dry soil and did not exceed the normative indicator 5 × 10^5 CFU/1 g abs. dry soil [14].

The number of SRB was 2.61 × 10^4 cells/1 g abs. dry soil that matches the characteristics of natural soil [17]. According to the TMC and the number of SRB in the studied soil, it can be suggested that it has a sufficiently high biological activity; the studied soil can be used to study the effects of microplastic accumulation in the environment.

Examples of samples of the studied materials removed from the soil and washed with sterile physiological NaCl solution are presented in Figure 1.



(**a**) (**b**)

Figure 1. The samples of materials removed from the soil and washed with sterile physiological NaCl solution: (**a**) Quartz sand; (**b**) Polypropylene microplastic.

In the biofilm of quartz sand and polypropylene such characteristic as TMC was at the same level—$1.59 \pm 0.27 \times 10^3$ and $1.55 \pm 0.19 \times 10^3$ CFU/cm^3, respectively. The number of SRB was 1.24×10^4 and 2.11×10^4 cells/cm^3, respectively, in the biofilm of quartz sand and polypropylene. Thus, the number of SRB in the biofilm on the surface of polypropylene was 1.7 times higher than in the biofilm on the surface of quartz sand. In this case, the obtained data are consistent with the results of Rogers et al. [18] regarding the greater number of microorganisms on plastic than on the surface of a material such as copper. It has also been shown that small and large polyethylene particles have significantly higher levels of bacteria than glass [19]. *Actinobacteria*, *Proteobacteria* and *Patescibacteria* have been established as the main microbiome of the "plastisphere" of polypropylene and expanded polystyrene [7].

Chen et al. [8] were established that the biomass of the biofilm is higher at 30th day than at 15th day. In generally inhabitation of the microplastic surface by microorganisms occurs within hours, and on the 14th day, the biofilm is fully formed [20]. The content of extracellular polymers in biofilms on microplastics also increases over time [8]. Microorganisms that concentrated on the surface of microplastics can biodegrade organic pollutants and degrade organic compounds, and play a significant role in the global carbon cycle and climate regulation. In addition, microplastics can affect the functioning of the soil microbial community [8].

4. Conclusions

The obtained results expand the understanding of biofilm formation processes on the surface of microplastics in soil and the participation of microorganisms in its biodegradation. It can be used in processes for removing harmful materials from soil.

Supplementary Materials: The conference presentation related to this paper can be downloaded as PDF using the link: https://sciforum.

Author Contributions: Conceptualization, N.T. and L.Z.; Methodology, N.T.; Validation, N.T.; Formal Analysis, N.T.; Investigation, N.T.; Resources, N.T.; Writing—Original Draft Preparation, N.T. and L.Z.; Writing—Review & Editing, N.T. and L.Z.; Visualization, N.T.; Supervision, N.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data used in this research are available upon request.

Acknowledgments: The authors are sincerely grateful to Barbara Urban-Malinga (Poland) for participating in the discussion of the research methodology.

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

References

1. Duis, K.; Coors, A. Microplastics in the aquatic and terrestrial environment: Sources (with a specific focus on personal care products), fate and effects. *Environ. Sci. Eur*. **2016**, *28*, 2. https://doi.org/10.1186/s12302-015-0069-y.
2. Barnes, D.K.; Galgani, F.; Thompson, R.C.; Barlaz, M. Accumulation and fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2009**, *364*, 1985–1998. https://doi.org/10.1098/rstb.2008.0205.
3. Chia, R.W.; Lee, J.Y.; Lee, M.; Lee, G.S.; Jeong, C.D. Role of soil microplastic pollution in climate change. *Sci. Total. Environ.* **2023**, *887*, 164112. https://doi.org/10.1016/j.scitotenv.2023.164112.
4. Dike, S.; Apte, S. Impact of microplastic pollution in terrestrial ecosystem on index and engineering properties of sandy soil: An experimental investigation. *Sci. Total. Environ.* **2023**, *887*, 164049. https://doi.org/10.1016/j.scitotenv.2023.164049.
5. Yurchenko, V.O.; Melnikova, O.G.; Ponomaryov, K.S.; Samokhvalova, A.I. Microplastics in bottom sediments of rivers in urbanized areas. In Proceedings of the International Scientific and Practical Internet Conference "Ecologically Sustainable Development of Urban Systems: Challenges and Solutions" KhNUUE named after O. M. Beketova, Kharkiv, Ukraine, 2–3 November 2021. Available online: http://eprints.kname.edu.ua/60576/1/C%D0%B1%D0%BE%D1%80%D0%BD%D0%B8%D0%BA21-134-136.pdf (accessed on 26 June 2023). (In Ukrainian)
6. Fortuna, M.; Borysovska, O. Assessment of water pollution by microplastic. *Collect. Sci. Work. Natl. Min. Univ.* **2021**, *65*, 195–206. Available online: http://znp.nmu.org.ua/index.php/en/archives/37-65en/441-65en18 (accessed on 26 June 2023). (In Ukrainian)
7. Kublik, S.; Gschwendtner, S.; Magritsch, T.; Radl, V.; Rillig, M.C.; Schloter, M. Microplastics in soil induce a new microbial habitat, with consequences for bulk soil microbiomes. *Front. Environ. Sci.* **2022**, *10*, 989267. https://doi.org/10.3389/fenvs.2022.989267.
8. Chen, Y.; Wang, X.; Wang, X.; Cheng, T.; Fu, K.; Qin, Z.; Feng, K. Biofilm Structural and Functional Features on Microplastic Surfaces in Greenhouse Agricultural Soil. *Sustainability* **2022,** *14*, 7024. https://doi.org/10.3390/su14127024.
9. McCormick, A.; Hoellein, T.J.; Mason, S.A.; Schluep, J.; Kelly, J.J. Microplastic is an abundant and distinct microbial habitat in an urban river. *Environ. Sci. Technol.* **2014**, *48*, 11863–11871. https://doi.org/10.1021/es503610r.
10. Tagg, A.S.; Oberbeckmann, S.; Fischer, D.; Kreikemeyer, B. Paint particles are distinct and variable substrate for marine bacteria. *Mar. Pollut. Bull.* **2019**, *146*, 117–124. https://doi.org/10.1016/j.marpolbul.2019.06.013.
11. Tkachuk, N.; Zelena, L.; Lukash, O.; Mazur, P. Microbiological and genetic characteristics of *Bacillus velezensis* bacillibactin-producing strains and their effect on the sulfate-reducing bacteria biofilms on the poly(ethylene terephthalate) surface. *EQ* **2021,** *32*, 119-129. https://doi.org/10.12775/EQ.2021.019.
12. Tkachuk, N.; Zelena, L. The Impact of Bacteria of the Genus *Bacillus* upon the Biodamage/Biodegradation of Some Metals and Extensively Used Petroleum-Based Plastics. *Corros. Mater. Degrad.* **2021**, *2*, 531–553. https://doi.org/10.3390/cmd2040028.
13. Yao, Y.; Rao, S.; Habimana, O. Active Microbiome Structure and Functional Analyses of Freshwater Benthic Biofilm Samples Influenced by RNA Extraction Methods. *Front. Microbiol*. **2021**, *12*, 588025. https://doi.org/10.3389/fmicb.2021.588025.
14. Yeromina, A.K.; Goncharova, N.G.; Sokolovska, I.A. *Ecology of Microorganisms: Teaching. Manual for Students of the 3rd Year of the Medical Faculty, Specialty "Laboratory diagnostics"*; ZSMU: Zaporizhzhia, Ukraine, 2013; 75p. Available online: http://library.zsmu.edu.ua/cgi/irbis64r_14/fulltext/Rejting/Er'ominaAK13_Ekolo_m.pdf (accessed on 26 June 2023). (In Ukrainian)
15. Furzikova, T.M.; Serhiychuk, M.G.; Vlasenko, V.V.; Shvets, Y.u.V.; Pozur, V.K. *Microbiology. Practicum*; Phytosocial Center: Kyiv, Ukraine, 2006; 210p. (In Ukrainian)
16. Tkachuk, N.; Zelena, L. Bacterial sulfidogenic community from the surface of technogenic materials *in vitro*: Composition and biofilm formation. *Biofouling* **2023**, *39*, 327–338. https://doi.org/10.1080/08927014.2023.2215694.
17. Abdulina, D.R.; Asaulenko, L.G.; Purish, L.M. Diversity of corrosive aggressive bacteria in soils of different biotopes. *Stud. Biol.* **2011**, *5*, 11–16. https://doi.org/10.30970/sbi.0501.111. (In Ukrainian)
18. Rogers, J.; Dowsett, A.B.; Dennis, P.J.; Lee, J.V.; Keevil, C.W. Influence of plumbing materials on biofilm formation and growth of Legionella pneumophila in potable water systems. *Appl. Environ. Microbiol.* **1994**, *60*, 1842–1851 https://doi.org/10.1128/aem.60.6.1842-1851.1994.
19. Parrish, K.; Fahrenfeld, N.L. Microplastic biofilm in fresh- and wastewater as a function of microparticle type and size class. *Environ. Sci. Water Res. Technol.* **2019**, *5*, 495–505. https://doi.org/10.1039/C8EW00712H.
20. Harrison, J.P.; Schratzberger, M.; Sapp, M.; Osborn, A.M. Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms. *BMC Microbiol.* **2014**, *14*, 232. https://doi.org/10.1186/s12866-014-0232-4.