

Proceeding Paper

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

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Abstract: The aim of this study was to investigate some chemical and microbiological characteristics of the soil and the surface biofilms 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 biofilms on the surface of the 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



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1. Introduction

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

The biological activity of microorganisms on polymeric materials that enter 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 biofilms of both polypropylene microplastics and quartz sand.

2. Materials and Methods

Particles of quartz sand (Poland) and primary pellets of polypropylene microplastics (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 the 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] and 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, 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 the Government Agency “Chernihiv Regional Center for Disease Control and Prevention” of the Ministry of Health of Ukraine (Chernihiv, Ukraine) following 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 biofilms on the surface of the 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 a 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 a 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 the 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 biofilms on the surfaces of quartz sand particles and polypropylene microplastics (pre-production pellets) were 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, and the number of bacteria in 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 was uncontaminated in terms of sanitary–hygienic and microbiological safety—the TMC was $1.26 \pm 0.15 \times 10^5$ 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, which 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.

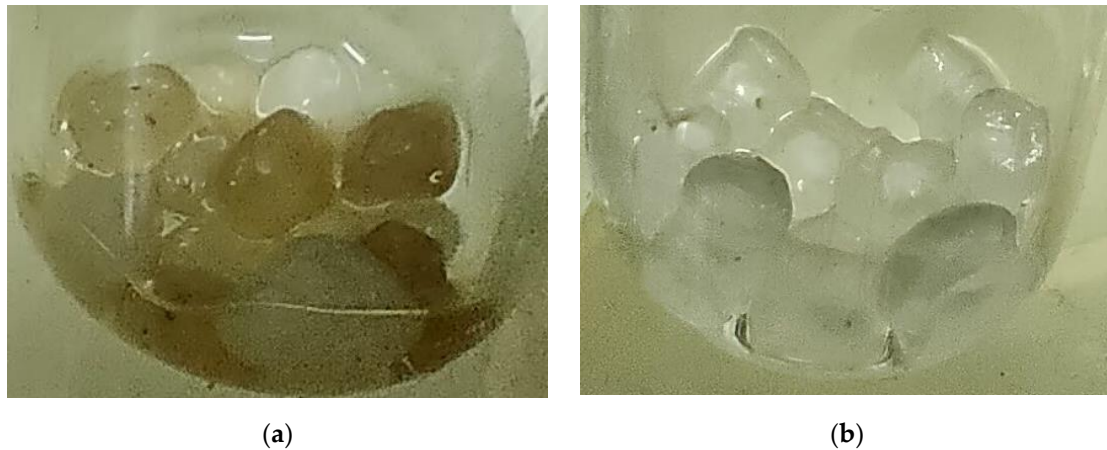


Figure 1. The samples of materials removed from the soil and washed with sterile physiological NaCl solution: (a) quartz sand and (b) polypropylene microplastic.

In the biofilms of quartz sand and polypropylene, the TMC was at the same level— $1.59 \pm 0.27 \times 10^3$ and $1.55 \pm 0.19 \times 10^3$ CFU/cm³, respectively. The numbers of SRB were 1.24×10^4 and 2.11×10^4 cells/cm³, respectively, in the biofilms 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] established that the biomass of the biofilm is higher on the 30th day than on the 15th day. In general, 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 concentrate on the surface of microplastics can biodegrade organic pollutants and degrade organic compounds, and they 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 their biodegradation. It can be used in processes for removing harmful materials from soil.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ASEC2023-15350/s1>, Conference Presentation: Some microbiological characteristics of the biofilm on the surface of pre-production pellets of polypropylene microplastics after short exposure in the soil.

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