Aquatic Toxicity of Polyethylene and Microcrystalline Cellulose Microbeads Used as Abrasives in Cosmetics

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Microplastics have been part of personal care products for years, but due to microplastic pollution, many companies have replaced microplastics with natural particles, such as microcrystalline cellulose. Although natural particles are considered more environmentally friendly, their ecotoxicological profile is unknown. In this context, the aim of this study was to compare the ecotoxicity of polyethylene and microcrystalline cellulose microbeads, both extracted from a cosmetic product. The effects of the two types of particles on the aquatic macrophyte *Lemna minor* and the crustacean *Daphnia magna*, as well as the bioadhesion of the particles to *Lemna minor* were evaluated. The results showed no significant effects of either particle on the specific growth rate, root length, and chlorophyll content of *Lemna minor*. The bioadhesion of both types of particles to the plant biomass was comparable. Furthermore, no significant effects were observed on the mobility and body length of *Daphnia magna*. Thus, the investigated polyethylene and cellulose microbeads showed no significant toxic effects on the tested organisms. However, due to the persistence of polyethylene in the environment, the use of polyethylene microbeads in cosmetics and personal care products should be avoided.

Keywords

cellulose particles, ecotoxicity, *Daphnia magna*, *Lemna minor*, microbeads, polyethylene

Introduction

Personal care products contain various ingredients, including small plastic particles called microbeads[1]. For many years, these particles have been used as cleansing or exfoliating agents in shower gels, toothpaste, nail polishes, and many others[1]. They are made from various polymers, most commonly from polyethylene (PE), but also polypropylene (PP), polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), nylons, and polyurethanes[1,2]. After use, microbeads are released into the sewage system and enter a wastewater treatment plant (WWTP) together with wastewater[3]. The removal efficiency depends on the type and design of a WWTP, but even with a high removal efficiency, many microbeads can still enter the environment. When microbeads reach the aquatic environment, those made of low density polymers (e.g. PE, PP) tend to float on the water surface, but they can also be in the water column or sediment due to simultaneous sorption of other organic matter and microorganisms[1]. Moreover, they can also interact with other pollutants, and consequently the toxic effects of microbeads could increase[4]. The negative effects of microbeads on various organisms have been widely investigated using different organisms, such as bacterium *Vibrio fischeri*[5], microalgae[6,7], duckweed *Lemna minor*[8,9], fish[10], crustacean *Daphnia magna*[11,12], and zebrafish *Danio rerio*[13]. For example, Kalčikova *et al.* showed that PE cosmetic microbeads of irregular shape did not affect the specific growth rate of *Lemna minor* or chlorophyll content[9]. However, they affected root length of duckweed. Namely, a significant reduction in root length was observed, which was attributed to the adsorption of microbeads on the root and its mechanical damage[9]. Furthermore, Rozman *et al.* concluded that the morphology of microbeads affects their impact on *Lemna minor*; a significant reduction in duckweed root length was observed for the particles with rough surface and sharp edges[14]. Similarly, Jemec Kokalj *et al.* reported an 18 % reduction in root length of *Lemna minor* after exposure to irregular PE particles with sharp edges isolated from a facial cleansing product[8]. However,

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there were no significant effects of the same particles on *Danio rerio* and *Daphnia magna*[8]. An *et al.* conducted a study in which they investigated the effects of synthetic PE fragments and commercial PE microbeads, and observed a size-dependent reduction in growth and reproduction of *Daphnia magna*[15]. This was associated with bioaccumulation of PE fragments in the gut, resulting in reduced food intake, body length, and number of offspring, and consequently higher mortality of *Daphnia magna*[15]. Jemec Kokalj *et al.* observed bioaccumulation of PE microbeads (from various commercial products) in the gut of *Daphnia magna* and *Artemia franciscana*[16]. The authors concluded that the uptake of the particles in the test organisms was size-dependent, but acute mortality was not observed. In addition, Canniff *et al.* reported bioaccumulation of PE microbeads in the size of 63–75 μm in the intestine of *Daphnia magna*[17]. Microbeads uptake increased with increasing concentrations of PE microbeads (25, 50, and 100 mg L^{-1}) and with exposure time (5 and 21 days), but without significant effects on *Daphnia magna* mortality[17]. Furthermore, survival and clearance rates of green mussels *Perna viridis* were not significantly affected after 21 days of exposure to polyvinyl-chloride (PVC) and red clay particles (at three concentrations of 1.5, 15, and 150 mg L^{-1}), but the lowest body condition index (providing information of organisms fitness and nutritional status) was found for a mussel exposed to PVC particles[18]. A significant difference in the fitness of the mussels was found after 21 days of exposure, suggesting that the toxic effects may be greater with longer exposure time, as was also reported in previous studies[18,19]. Harris and Carrington reported a 62 % inhibition of the clearance rate of the mussel *Mytilus trossulus* when exposed to a high concentration of PE microbeads > 1250 particles mL^{-1} [20], while silt particles had no effect on the clearance rate at the same tested concentrations[20]. Authors suggested that microbeads may decrease clearance rate at high concentrations due to unique surface properties that interfere with the filtration process. Generally, mussels clarify water, and this reduction in clearance rate reduces their ability to filter turbid water and use energy available from food for processes such as growth, reproduction, and metabolism. The reduction in water clarity and benthic-pelagic coupling can affect the entire ecosystem[20].

Due to their negative impacts and the high number of microbeads released into the environment (e.g., it is estimated that approximately 8 trillion microbeads enter aquatic ecosystems every day in the United States[21]), the use of microbeads in cosmetics and personal care products has been largely restricted. In 2018, eight of the world's 192 countries (Canada, France, Italy, the Republic of Korea, New Zealand, Sweden, the United Kingdom of Great Britain and Northern Ireland, and the United States of America) introduced a mandatory ban on microbeads through national laws or regulations[22]. In addition, the European Union (EU) announced that the European Commission has begun the process of banning intentionally added microplastics as part of its European Strategy for Plastics[21,22]. Therefore, plastic microbeads have been replaced by microbeads made from natural materials, such as microcrystalline cellulose[23]. However, the effects of these particles on organisms are unknown.

In this context, the aim of this study was to compare the effects of polyethylene and microcrystalline cellulose microbeads on the two test organisms duckweed *Lemna minor* and crustacean *Daphnia magna*. They were selected as the model aquatic producer and consumer, respectively. They are both extensively used in the ecotoxicity studies, because they are simple to maintain in the laboratory, they have relatively rapid growth and reproduction, and they are sensitive to particle exposure[24,25].

Materials and methods

Polyethylene and microcrystalline cellulose microbeads

The particles used were polyethylene (PE) microbeads and microcrystalline cellulose microbeads (further named as cellulose microbeads) extracted from a commercially available facial scrub. Both types of microbeads were extracted from the same product a few months apart, as the manufacturer in the meantime replaced PE microbeads with cellulose microbeads. The presence of PE and cellulose microbeads was indicated in the list of ingredients on the package. To extract the particles from the cosmetic product, approximately 20 mL of the product was added to 500 mL of warm deionised water (40 ± 2 °C), according to Kalčíková *et al.*[9] Microbeads were separated from the solution by filtration using cellulose 4–12 μm filters (Whatman™, Germany). Afterwards, microbeads were washed several times with deionised water to remove potentially remaining ingredients from the surface, and dried at 40 ± 2 °C overnight.

Chemical characterization of microbeads was done by Fourier-transform infrared spectroscopy (FTIR, SpectrumTwo FT-IR, PerkinElmer, UK) in an ATR mode at the wavenumber 4000–450 cm^{-1} (the resolution was 2 cm^{-1}, 10 scans). Background and ATR correction of the spectra was used. The morphology characteristics of the microbeads were studied by field-emission scanning electron micros-

copy (FE-SEM, Zeiss Ultra plus). Prior to the analysis, microbeads were coated with a thin Au/Pd layer to increase the electrical conductivity of the samples. Laser diffraction analyser S3500 Bluwave (Microtrac, Germany) was used to determine the mean size of the microbeads. The mean number of microbeads per unit mass was determined by weighing approximately 1 mg of particles, and counting them under the stereo microscope SMZ-171 (Motic, China). The procedure was repeated ten times to include at least 1000 particles in the analysis.

Experimental design

In order to investigate the ecotoxicity impact of PE microbeads and cellulose microbeads, duckweed *Lemna minor* and crustacean *Daphnia magna* were used as test organisms. They both originated from a permanent laboratory culture that is cultivated at the University of Ljubljana, Faculty of Chemistry and Chemical Technology (Slovenia). The tested concentration of PE microbeads and cellulose microbeads was 5 000 particles mL^{-1} (being 26 mg L^{-1} of PE microbeads and 104 mg L^{-1} of cellulose microbeads – based on the number of particles per unit mass). The concentration was chosen comparable to those reported in freshwaters[26,27] and therefore, it can be considered environmentally relevant.

The ecotoxicity test with duckweed *Lemna minor* was performed in 100-mL beakers containing 50 mL Steinberg medium[28] (consisting of 20 mL L^{-1} of solutions 1 (17.50 g L^{-1} KNO_3, 4.50 g L^{-1} KH_2PO_4, 0.63 g L^{-1} K_2HPO_4), 2 (5 g L^{-1} $MgSO_4 \cdot 7H_2O$), and 3 (14.75 g L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$) and 1 mL L^{-1} of solutions 4 (120 mg L^{-1} H_3BO_3), 5 (180 mg L^{-1} $ZnSO_4 \cdot 7H_2O$), 6 (44 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$), 7 (180 mg L^{-1} $MnCl_2 \cdot 4H_2O$), and 8 (760 mg L^{-1} $FeCl_3 \cdot 6H_2O$ and 1500 mg L^{-1} $EDTA \cdot 2H_2O$)), and 10 fronds of duckweed with removed roots were added in each beaker. Each treatment (control, with PE microbeads, and cellulose microbeads) was replicated four times. The experiment was performed in a climate chamber at 24 ± 2 °C and high humidity (> 70 %) with a photoperiod of 16/8 h (light/dark) during an exposure time of 7 days. After the incubation period (7 days), the number of fronds was determined, and the specific growth rate was calculated (eq. 1):

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t} \quad (1)$$

where μ represents specific growth rate of *Lemna minor* (d^{-1}), N_t number of fronds of *Lemna minor* at the end of the experiment (/), N_0 number of fronds of *Lemna minor* at the beginning of the experiment (/), and t exposure time (d).

Mean root length of duckweed was determined by measuring root length of 10 randomly selected plants in each beaker with millimetre paper. Furthermore, for determination of the chlorophyll *a* content, approximately 20 mg of fresh plant was weighed and homogenized (ground in a mortar and pestle) in the dark with 95 % ethanol, and incubated at –18 ± 2 °C for 24 h. Absorbance of the supernatant was measured spectrophotometrically at wavelengths of 664.2 and 648.6 nm (Cary 50 UV-Vis spectrophotometer, Agilent Technologies, USA), and the concentration of chlorophyll *a* was calculated according to Lichtenthaler[29].

The ecotoxicity test with crustacean *Daphnia magna* followed the standard procedure with some modifications[30]. The experiment was performed in 100-mL beakers containing 50 mL M4 medium[30] (consisting of 4 mL L^{-1} of solution 1 (73.52 g L^{-1} $CaCl_2 \cdot 2H_2O$), 1 mL L^{-1} of solutions 2 (123.30 g L^{-1} $MgSO_4 \cdot 7H_2O$), 3 (5.8 g L^{-1} KCl), and 4 (64.8 g L^{-1} $NaHCO_3$), 0.1 mL L^{-1} of solutions 5 (3.61 g L^{-1} $MnCl_2 \cdot 4H_2O$, 3.06 g L^{-1} LiCl, 0.71 g L^{-1} RbCl, 1.52 g L^{-1} $SrCl_2 \cdot 6H_2O$, 0.17 g L^{-1} $CuCl_2 \cdot 2H_2O$, 0.13 g L^{-1} $ZnCl_2$, 0.10 g L^{-1} $CoCl_2 \cdot 6H_2O$), and 6 (750 mg L^{-1} thiamine hydrochloride, 10 mg L^{-1} cyanocobalamin, 7.5 mg L^{-1} biotin), 0.5 mL L^{-1} of solutions 7 (548 mg L^{-1} $NaNO_3$, 5719 mg L^{-1} H_3BO_3, 32 mg L^{-1} NaBr, 126 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 6.5 mg L^{-1} KI, 4.38 mg L^{-1} Na_2SeO_3, 1.15 mg L^{-1} NH_4VO_3) and 8 (286 mg L^{-1} KH_2PO_4, 368 mg L^{-1} K_2HPO_4), 0.2 mL L^{-1} of solution 9 (21.48 mg L^{-1} Na_2SiO_3), and 5 mL L^{-1} of solution 8 (500 mg L^{-1} $Na_2EDTA \cdot 2H_2O$, 199 mg L^{-1} $FeSO_4 \cdot 7H_2O$)). A seven-day-old *Daphnia magna* specimen was added to each beaker, and each treatment (control, PE microbeads, and cellulose microbeads) was replicated ten times. There were two tested setups – with and without feeding of *Daphnia magna* with algae (*Spirulina* sp.). The experiment was performed at room temperature (22 ± 1 °C) and with a photoperiod of 16/8 h (light/dark). The incubation time was 3 days, and the effects of particles on immobility and body length were monitored. The latter was measured with a stereo microscope (SMZ-171, Motic).

Bioadhesion to *Lemna minor*

The adhesion of microbeads to *Lemna minor* biomass was also determined by digestion of plant biomass and counting of recovered microbeads. Briefly, approximately 20 mg of fresh plant from each replicate was washed with deionised water and weighed. Afterwards, the Fenton oxidation was used for degradation of plants according to the procedure described in Rozman *et al.*[31] Briefly, 2 mL of 0.015 g mL^{-1} of $FeSO_4 \cdot 7H_2O$ (with 3 mL L^{-1} of H_2SO_4 (97 %)) and 2 mL of 30 % H_2O_2 were added to the plant biomass, and after 24 h of degradation at room temperature, the solution was filtered using cellulose 0.22 μm sterile filters (S-Pak filter, Merck

Millipore). Filters were dried at room temperature for 24 h, and remaining particles were counted using the stereo microscope. In addition, the impact of Fenton oxidation on PE microbeads and cellulose microbeads was investigated. PE microbeads and cellulose microbeads were weighed in ten replicates (approximately 30 mg), digested with Fenton oxidation, processed as described previously, and reweighed. The mass of PE and cellulose microbeads had reduced by 4.4 ± 0.8 % and 2.3 ± 1.4 %, respectively, indicating that the impact of Fenton oxidation was negligible for both microbeads.

Results and discussion

Personal care and cosmetic industry use various polymers as important ingredients[32]. They can be divided into three groups: synthetic, semi-synthetic, and natural polymers[32]. Due to the ubiquitous presence of microplastics in the environment, manufacturers have begun to replace synthetic polymers (e.g., PE) with semi-synthetic ones (e.g., microcrystalline cellulose). This replacement is desirable according to eco-friendliness, and on the other hand, they are still able to achieve the same cleaning efficiency as particles made from synthetic polymers[32]. However, their effects on various aquatic organisms have not been often studied.

Characterization of polyethylene and microcrystalline cellulose microbeads

The chemical composition of the particles was determined by FTIR spectroscopy. The FTIR spectra of PE and cellulose microbeads are presented in Fig. 1. The FTIR spectrum for PE microbeads (Fig. 1a) confirmed that microbeads were low-density PE, as peaks at 2915 cm^{-1} and 2848 cm^{-1} corresponded to C–H stretching, CH_2 bending peaks occurred at 1472 cm^{-1} and 1463 cm^{-1}, CH_3 bending peak was noticed at 1377 cm^{-1}, and CH_2 rocking peaks at 729 cm^{-1} and 719 cm^{-1} [33]. Cellulose microbeads exhibited a peak at 3336 cm^{-1}, which corresponded to surface hydroxylation O–H stretching mode. Asymmetrical stretching vibration of C–H in a pyrenoid ring occurred at 2917 cm^{-1}, while stretching and bending of the surface hydroxyls was noticed at 1641 cm^{-1}. Peaks at 1051 cm^{-1} and 1027 cm^{-1} corresponded to the C–O functional group (Fig. 1b)[34].

The shape and morphology of the microbeads were studied by FE-SEM, and are shown in Fig. 2. Both microbeads had uniform spherical shape; however, surface of PE microbeads was smooth with some grooves (Fig. 2a), while cellulose microbeads had highly irregular surface, as shown in Fig. 2b. Moreover, the mean size of PE and cellulose microbeads was 159 ± 91 μm and 296 ± 45 μm, respectively, and the number of PE microbeads per unit mass was 185 particles mg^{-1} and for cellulose microbeads 48 particles mg^{-1}.

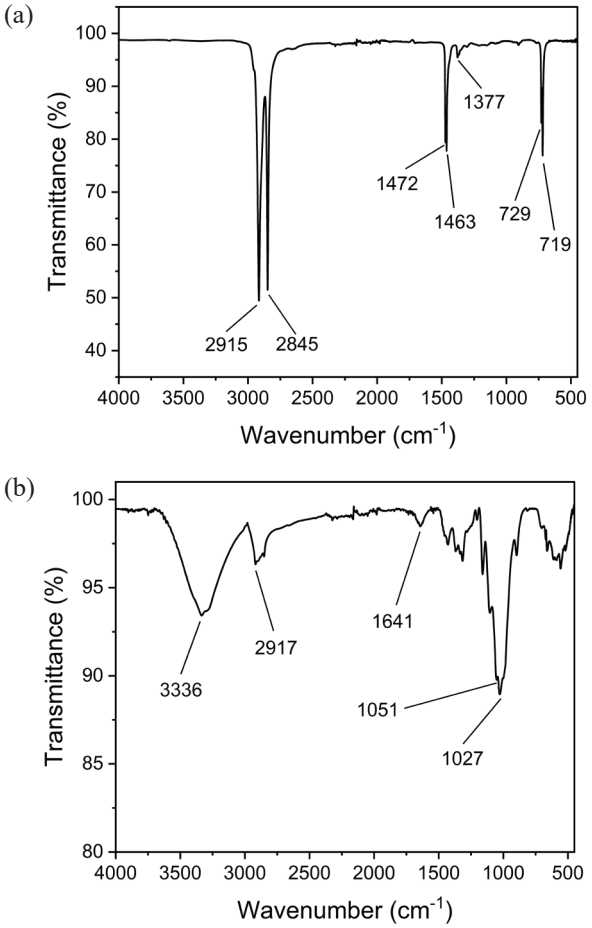

Fig. 1 – *FTIR spectrum of PE microbeads (a) and cellulose particles (b).*

Ecotoxicological effects

Table 1 presents the results of ecotoxicity tests with *Lemna minor* and *Daphnia magna* for PE and cellulose microbeads. Based on the results, no effect of either particle on the specific growth rate and root length of *Lemna minor* was observed (Table 1). Similarly, chlorophyll *a* content of *Lemna minor* was also not affected by PE or cellulose microbeads. These results are in accordance with results of Kalčikova *et al.,* when the growth rate and chlorophyll *a* content in *Lemna minor* was not affected after exposure to PE microbeads[9]. In contrast, some previous studies have shown significant reduction in the root length of duckweed when exposed to PE microbeads[8,9,14]. However, the impact was observed only when particles were irregularly shaped and had sharp edges that damaged root cells[14]. In our study, both particles were spherical, therefore significant abrasion and thus effects on

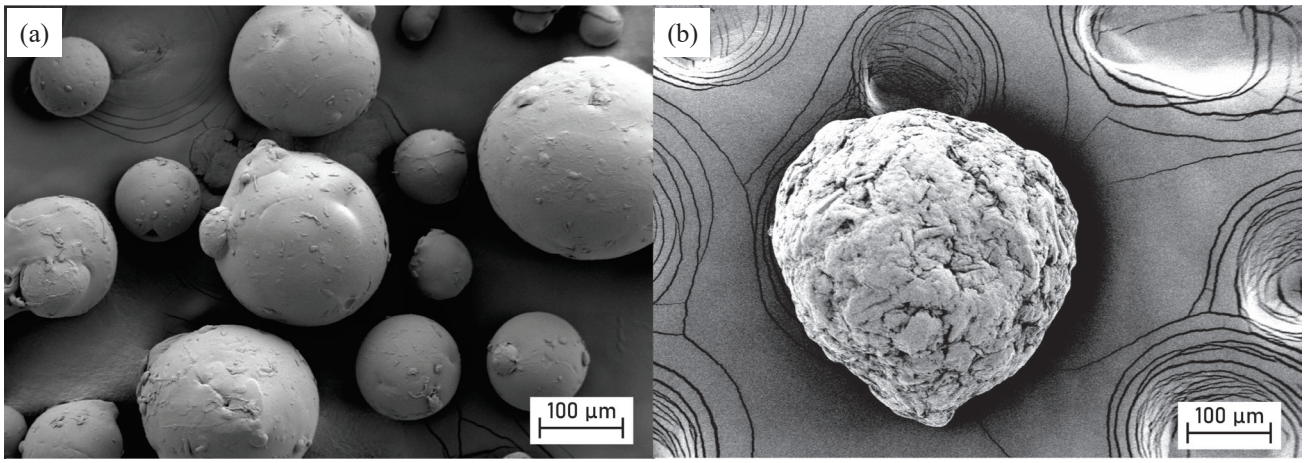

Fig. 2 – *FE-SEM images of PE microplastics (a) and cellulose particles (b)*

Table 1 – *Effects of polyethylene (PE) and cellulose microbeads on Lemna minor and Daphnia magna. Results are expressed as mean value ± SD.*

Treatment	*Lemna minor*			*Daphnia magna*			
	Specific growth rate / d^{-1}	Root length / mm	Chl *a* / mg C*a* g_{FW}^{-1}	Mobility / %		Body length / mm	
Control	0.28 ± 0.00	12.25 ± 0.82	0.12 ± 0.01	100*	100**	1.84 ± 0.32*	1.45 ± 0.18**
PE microbeads	0.27 ± 0.01	14.50 ± 0.36	0.18 ± 0.03	70*	90**	1.81 ± 0.48*	1.46 ± 0.11**
Cellulose microbeads	0.27 ± 0.01	15.23 ± 1.10	0.13 ± 0.01	90*	100**	1.87 ± 0.20*	1.47 ± 0.16**

FW = fresh weight; *conditions without feeding; **conditions with feeding; / – not observed

roots were not expected. Similarly, Mateos-Cárdenas *et al.* observed no impact on root length of *Lemna minor* when plant was exposed to spherical PE particles[35].

After exposure of the seven-day-old *Daphnia magna* to PE microbeads and cellulose microbeads, the mobility and body length were monitored. No significant change in body length of *Daphnia magna* was observed, as the body length of *Daphnia magna* exposed to PE and cellulose microbeads was comparable to that of the control organisms. Frydkjær *et al.* showed that microparticles with irregular shape exhibit more pronounced inhibitory effects on *Daphnia magna* than microparticles with a regular shape[36]. In general, *Daphnia magna* is a filter feeder that feeds on particles within a size range of 1–70 µm[37]; however, the ingestion of particles depends on various factors, such as size and shape of microbeads, but also on the age of the organisms[36,37]. In this study, seven-day-old *Daphnia magna* were able to ingest some of the smaller microbeads, but a large portion of the particles remained in the medium because they were too large to be ingested. Under the conditions without feeding, negative effect on mobility was noted in the treatment with PE microbeads; therefore, it is plausible that *Daphnia magna* could ingest more microbeads compared to those that were fed by algae. After ingestion, microparticles could accumulate in organisms (e.g., in the digestive system or in gills), which leads, in some cases, to a false sense of satiety, and consequently death[38]. Furthermore, irregularly shaped particles (e.g., fragments and fibres) are excreted more slowly than microbeads[35] and can even become lodged in the digestive system[39]. In addition, toxicity is higher when the size of microparticles decreases and the concentration of microplastics increases[40]. In addition, Pawlak *et al.* compared the effects of PS and natural microbeads made of silica with a size of 1, 3, and 6 µm on the rotifer *Brachionus calyciflorus* in different exposure times[19]. After 24 hours, no toxic effect on the survival of rotifers was observed for any of the particles studied. After 96 hours, the survival rate of *Brachionus calyciflorus* decreased in treatments containing 6 µm PS and all three sizes of studied silica microbeads[19]. Moreover, reproduction of rotifers decreased after 96 hours of exposure to all sizes of PS microbeads, but for 3 and 6 µm of silica particles the reproduction was not deteriorated. This suggests that microbeads ingestion has a greater effect on the reproductive rate of freshwater zooplankton than on their survival[19].

In summary, the effects of synthetic and natural microbeads can be similar, which means that natural microbeads should also be studied in comparison to synthetic microbeads. However, the toxic ef-

fects of microparticles on different test organisms may differ due to the type, size, concentration, bioavailability, or shape of the particles studied, the characteristics of the test organisms, the parameters observed, the experimental design (e.g., conditions with or without feeding test organisms), and/or the exposure time.

Bioadhesion to *Lemna minor*

The bioadhesion of PE and cellulose microbeads to *Lemna minor* was monitored, and the results showed that the bioadhesion of both types of particles was comparable: 0.042 ± 0.000 of PE microbeads and 0.041 ± 0.001 of cellulose microbeads per mg biomass adhered to *Lemna minor*. This confirmed the interactions between microbeads and plant biomass, but no significant effect was observed (Table 1) on leaves and roots of *Lemna minor*. It is plausible that microbeads adhered only weakly, as also demonstrated by Mateos-Cárdenas *et al.*[35] On the other hand, other types of microplastics, such as microfibers can adhere strongly due to their elongated shape[41]. Rozman *et al.* showed that 75 % of PE microbeads (in the form of fragments) adhered weakly to *Lemna minor* biomass[24]. The interactions of microbeads and plants can have important consequences. For example, Mateos-Cárdenas *et al.* reported the transfer of microbeads attached to *Lemna minor* biomass to the freshwater amphipod *Gammarus duebeni* after consumption of *Lemna minor*[35]. This is a clear indication of possible transfer of microbeads through the food chain.

Conclusions

In the past, plastic microbeads were mostly used in personal care products. Nowadays, cosmetic industry has mostly replaced them with natural-based particles, such as cellulose microbeads. In this study, we evaluated the effects of both particles (PE and cellulose microbeads) on two aquatic organisms, *Lemna minor* and *Daphnia magna*. The results showed that PE and cellulose microbeads in environmentally relevant concentrations had no significant toxic effect on both organisms. However, cellulose is readily degradable[42,43] whereas PE is persistent, and its degradation lasts for decades[44,45]. PE also has several direct and indirect negative effects on the environment, e.g., the presence of PE in soil increases the mobility of organic contaminants[46], alters soil properties such as aggregation and pore size, subsequently increasing water evaporation[47], fosters microbial growth in lakes[48], and affects microbial activity in sediment[49]. Therefore, the release of PE into the natural environment should be minimised.

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A list of abbreviations

PE – polyethylene
PP – polypropylene
PET – polyethylene terephthalate
PMMA – polymethyl methacrylate
WWTP – wastewater treatment plant
FTIR – Fourier-transform infrared spectroscopy
FE-SEM – field-emission scanning electron microscopy
PVC – polyvinyl-chloride

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