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Impact of polyethylene microbeads on the floating freshwater plant duckweed *Lemna minor*☆

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ABSTRACT

Microplastics (MP), small plastic particles below 5 mm, have become one of the central concerns of environmental risk assessment. Microplastics are continuously being released into the aquatic environment either directly through consumer products or indirectly through fragmentation of larger plastic materials. The aim of our study was to investigate the effect of polyethylene microbeads from cosmetic products on duckweed (*Lemna minor*), a freshwater floating plant. The effects of microbeads from two exfoliating products on the specific leaf growth rate, the chlorophyll *a* and *b* content in the leaves, root number, root length and root cell viability were assessed. At the same time, water leachates from microbeads were also prepared to exclude the contribution of cosmetic ingredients on the measured impacts. Specific leaf growth rate and content of photosynthetic pigments in duckweed leaves were not affected by polyethylene microbeads, but these microbeads significantly affected the root growth by mechanical blocking. Sharp particles also reduced the viability of root cells, while the impact of microbeads with a smooth surface was neglected. It was concluded that microbeads from cosmetic products can also have negative impacts on floating plants in freshwater ecosystems.

1. Introduction

Microplastics (MP), defined as small plastic particles below 5 mm (Thompson et al., 2004), have become one of the central concerns of environmental risk assessment. These small particles are continuously being released into aquatic environments either directly through consumer products or indirectly through ultraviolet radiation, physical forces, as well as hydrolysis, biological degradation and disintegration of larger plastic material (Mattsson et al., 2015). During the last 5 years, MP have been repeatedly discovered in marine habitats (Cole et al., 2011), various freshwater ecosystems (Baldwin et al., 2016) and even in remote freshwater environments, such as mountain lakes (Imhof et al., 2013).

Personal care and cosmetic products (lotions, soaps, facial and body scrubs and toothpastes) are known to be considerable sources of MP in freshwater systems (Baldwin et al., 2016; Bhattacharya, 2016; Carr et al., 2016). Everyday use of personal care and cosmetic products releases MP directly into wastewater. Fendall and Sewell (2009) analysed the microbeads content in four water-based facial cleaners containing polyethylene. The microbeads contained in all brands of facial cleansers showed a variety of irregular shapes from ellipses, ribbons, and threads, to completely irregular fragments. Microbeads in the facial cleansers showed a wide size range with few larger than 1 mm and the majority of particles smaller than 0.5 mm.

Even back in 2009, Fendall and Sevell had pointed out that MP can possibly pass through waste water treatment plants (WWTPs) and enter freshwaters (Fendall and Sewell, 2009). Recent studies have revealed that the majority of particles are retained by the WWTPs (Carr et al., 2016), however WWTPs are still considered to play an important role in environmental MP pollution (Mintenig et al., 2017). Furthermore, MP can enter freshwater in the case of overflow from sewage in case of heavy downpour and runoff from sewage-based fertilizer deposited on agricultural land (Bhattacharya, 2016). The majority of MP identified in different effluents within the WWTP had a profile similar to the blue polyethylene particles present in toothpaste formulations (Carr et al., 2016). Similarly, a report on the monitoring of meso-to micro-sized floating litter items in four large European rivers (Rhine, Dalålven, Danube and Po) revealed that ***polyethylene*** was the most

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prevalent material in all rivers (SFRA0025, 2015).

A number of studies have already demonstrated the adverse effects of MP on various marine and freshwater invertebrates and vertebrates (Bouwmeester et al., 2015; Mattsson et al., 2015). Most of the data are available for polystyrene MP, while data for polyethylene derived MP are rather scarce (but see e.g. Mazurais et al., 2015). In addition, adverse effects on plants are significantly understudied, with most of the data available only for phytoplankton. For example, Bhattacharya et al. (2010) showed enhanced adsorption of polystyrene nanospheres onto *Chlorella* and *Scenedesmus sp.* resulting in adverse effects on photosynthesis and an increase in the production of reactive oxygen species (ROS). Polystyrene nanospheres reduced the growth of marine diatom *Thalassiosira pseudonana*, flagellate *Dunaliella tertiolecta* and *Chorella vulgaris* (Sjollema et al., 2016). However, to our knowledge, no other data on the potential interaction of ***MP with higher aquatic plants*** are currently available.

Low density MP, such as polyethylene microbeads with a specific density <1 g cm^{-3}, are abundant in the water surface microlayer (Eerkes-Medrano et al., 2015) and thus can affect floating plants that have their entire bottom surface and roots in permanent contact with the water surface. Furthermore, floating plants may also possibly aggregate MP during their movement on the water surface (Prokin et al., 2015). Freshwater MP are often retained in lakes and reservoirs (Zhang et al., 2015), both of which are typical habitats for floating duckweeds of the Lemnaceae family. Duckweeds are very important plants in aquatic ecosystems: they represent food for fish and waterfowl and serve as a nursery habitat for many species, providing both protections from predators and enhanced feeding opportunities (Van Hoeck et al., 2015). Interactions of MP with floating plants have not been investigated so far and more knowledge is needed due to the crucial role of duckweeds in aquatic ecosystems.

The aim of our study was to investigate the effect of polyethylene cosmetic microbeads on the duckweed *Lemna minor*. The effects of MP on the specific leaf growth rate, the leave chlorophyll content, root number, root length and root cell viability were assessed.

2. Materials and methods

2.1. Microplastics extraction and preparation

Two exfoliating products (A and B) containing polyethylene were chosen for the experiment. In order to extract microbeads, cosmetic products were dissolved in deionized water and then filtered *via* vacuum filtration using Whatman™ filter paper (pore size 4–12 μm). The retained microbeads were washed several times by filtration of deionized water through the filter paper to remove any remaining ingredients from the cosmetic products. Microbeads were carefully washed from the filter into glass beakers and dried overnight in a laboratory dryer (Kambič, Slovenia) at 60 °C. A stock solution of microbeads (100 mg L^{-1}) was prepared by dispersion of 10 mg of microbeads in 100 mL of Steinberg medium (ISO 20079, 2005) and mixing with a magnetic stirrer for 5 min.

2.2. Duckweed Lemna minor

Duckweed *Lemna minor* L. originated from a laboratory culture of the Institute of Chemistry and Technology of Environmental Protection (Faculty of Chemistry, Brno University of Technology, Czech Republic). In our laboratory, it has been successfully cultivated in Steinberg medium (ISO 20079, 2005) under controlled conditions (temperature 23 ± 2 °C, photoperiod 16/8 h) for more than seven years.

2.3. Experimental design

The experiment was conducted in 6-well culture plates (TPP®, Switzerland) and each well was filled with 10 mL of test solution. Concentration of microbeads in the experiment were 0, 10, 50, and 100 mg L^{-1}. Concentrations were prepared by dilution of the microbeads stock solution by Steinberg medium. These concentrations were chosen based on the concentration range used in previous MP toxicity studies with freshwater crustaceans (Besseling et al., 2014). Each experiment included 3 replicates of the same exposure concentration and each experiment was repeated twice. The initial number of fronds was six, leaving more than 50% of surface area for further growth. The experiment was performed in a climate test chamber at 24 ± 2 °C and high humidity (>70%) to minimize evaporation of the test media. All treatments were illuminated by daylight fluorescent lamps with a photoperiod of 16/8 h (light/dark) at a light intensity 3507 ± 529 lux (mean ± SD, n = 10) at plant level. The experiment proceeded for seven days and at the end of the experiment the number of fronds was counted. Afterwards, the duckweed was gently collected and washed several times with demineralized water to remove attached microbeads from the fronds and roots for further analyses.

2.4. Photosynthetic pigment determination

Photosynthetic pigments were determined according to Radić and Pevalek-Kozlina (2010), with some modifications. Approximately, 20 mg of fresh plant material per treatment was used for analyses of photosynthetic pigments (chlorophyll *a and b*). Samples were homogenized in the dark with 95% ethanol (v/v) and stored in a freezer at - 28 °C. Absorbance of the supernatant was read at 664.2 and 648.6 nm (Cary 50 UV-VIS, Varian).

2.5. Measurement of the root length

Duckweed plantlets were arranged in Petri dishes together with millimeter paper and photographed with a Canon 1000D digital camera and EF-S 60 mm macro lens (Canon, Japan). Root length was measured on acquired images in Fiji (Schindelin et al., 2012).

2.6. Assessment of root cell viability (Evans Blue staining)

Duckweed was transferred from the tested solutions to vials containing 0.05% Evans Blue in distilled water. Staining was performed for 10 min on a rotary shaker at 60 rpm, followed by 3 × 5 min washes in distilled water to remove unabsorbed stain. Roots were observed and photographed with an Axioskop 2 MOT microscope, Plan Neofluar 10 × /0.30 objective and AxioCam MRc camera (Zeiss, Germany). As a positive control for assessment of root cell viability, plants were treated with 0–100 mg L^{-1} $K_2Cr_2O_7$ in Steinberg medium for seven days, followed by Evans Blue staining as above.

2.7. Effect of microbeads' water leachates

Additional treatment was prepared to assess the possible impact of cosmetic ingredients leached from microbeads. Microbeads (100 mg L^{-1}) were dispersed in the Steinberg medium and incubated under the same experimental conditions as used in the tests with duckweed. After seven days of incubation, microbeads in Steinberg medium were filtered through 0.45 μm filter and the undiluted filtrate (microbeads leachate) was used for the test with duckweed as described above (Chapter 2.3.). The impact of leached cosmetic ingredients on leaves and roots were assessed by measuring the specific leaf growth rate, root length and root cell viability.

2.8. Data analysis

The average specific leaf growth rate for the period of seven days is calculated according to (ISO 20079, 2005) as follows:

$$\mu = \frac{ln(N_j) - ln(N_i)}{t}$$

where μ (d^{-1}) is the average specific leaf growth rate, *Nj* (/) is the number of fronds at the end of the experiment, *Ni* (/) is the number of fronds at the beginning of the experiment and *t* (d) is a time period of exposure (seven days).

Chlorophyll (Chl) *a* and *b* content was calculated according to Lichtenthaler (1987). The results are given as mg of chlorophyll per gram of fresh weight (Chl*a* or Chl*b*·g^{-1}_{FW}).

The statistical significances of the differences between the control and exposed groups were assessed by using Mann-Whitney *U* test, where differences were considered significant if $p < 0.05$ using OriginPro 8.0 software (OriginLab Corp., Northampton, MA, USA).

3. Results

3.1. Microbead characteristics

Microbeads were extracted from two cosmetic products labeled as A and B. Both products were body scrubs and contained white particles of irregular shapes (Fig. 1). The B product contained also blue particles. According to the producers, the microbeads in both products were made of polyethylene. It was also confirmed by IR analysis (Kalčikova and Žgajnar Gotvajn, 2016). Microbeads from the A product were within the size range of 30 μm–600 μm (mean particle size 71.30 ± 34.29 μm, n = 3), while microbeads from the B product were between 40 μm and 400 μm (mean particle size 96.00 ± 69.99 μm, n = 3). The A and B products contained 853 and 625 particles per mg of microbeads, respectively (Kalčikova and Žgajnar Gotvajn, 2016).

3.2. Effect of microbeads on leaves

After seven days of incubation none of the treatments affected the specific leaf growth rate of duckweed leaves. All concentrations (10, 50, and 100 mg L^{-1}) of microbeads A caused less than 10% inhibition in comparison to control (5%, 8%, and 5% respectively) and similar low effect was observed for the same concentrations of microbeads B (3%, 8%, and 6% respectively). No significant reduction of photosynthetic pigment concentration (chlorophyll *a and b*) in comparison to controls was found in any of the investigated treatments (Fig. 2).

3.3. Effect of microbeads on roots

None of the tested concentrations of microbeads A and B (10, 50, and 100 mg L^{-1}) significantly affected the number of roots in comparison to controls (Fig. 3A). However, all concentrations of both microbeads caused a significant decrease in the length of duckweed roots (Fig. 3B). The mean root length in the case of control, 10, 50 and 100 mg L^{-1} was 3.13, 2.82, 2.34 and 2.37 cm (n = 15), respectively, in the case of microbeads A and 3.58, 3.01, 2.99, and 3.00 cm (n = 20), respectively, in the case of microbeads B.

The negative impact of microbeads on roots has been also confirmed by staining of roots with Evans blue dye (Fig. 4). The presence of microbeads compromised plant cell membrane integrity resulting in a bluish color of the roots (Fig. 4). When compared to a standard compound $K_2Cr_2O_7$, all tested concentrations of microbeads A (10, 50 and 100 mg L^{-1}) caused an effect comparable to 10 mg L^{-1} of $K_2Cr_2O_7$.

On the other hand, all concentrations of microbeads from the B

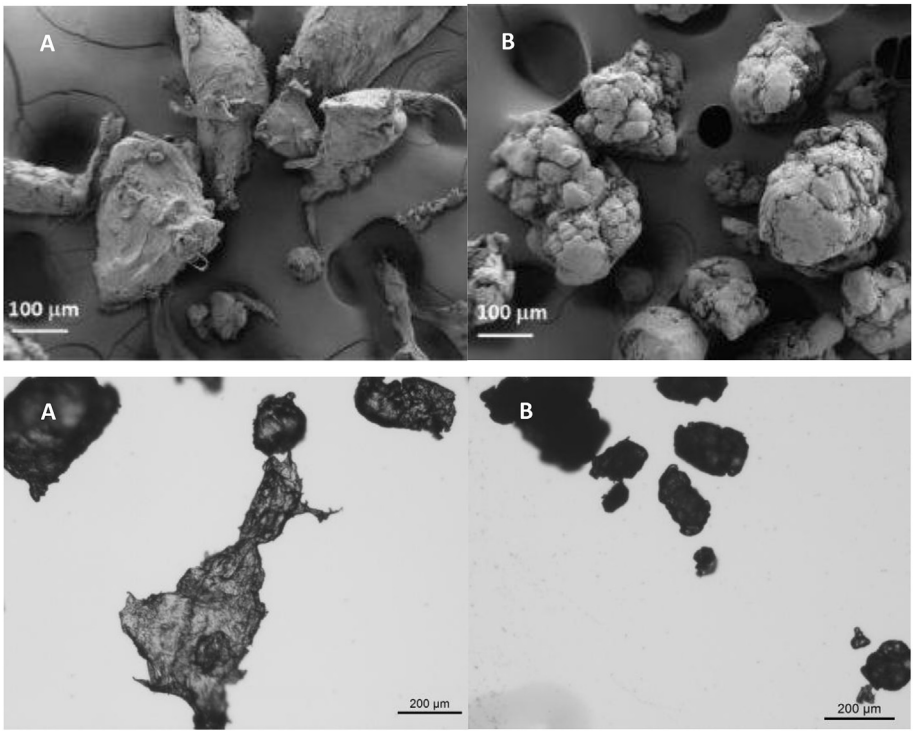

Fig. 1. Irregular shapes of A microbeads and B microbeads. Upper pictures are from a field emission scanning electron microscope (SEM) Supra 35VP (Carl Zeiss) (Kalčikova and Žgajnar Gotvajn, 2016), while lower pictures are from a light microscope (Leica MZFLIII, Leica microsystems, Germany, 4 x magnification).

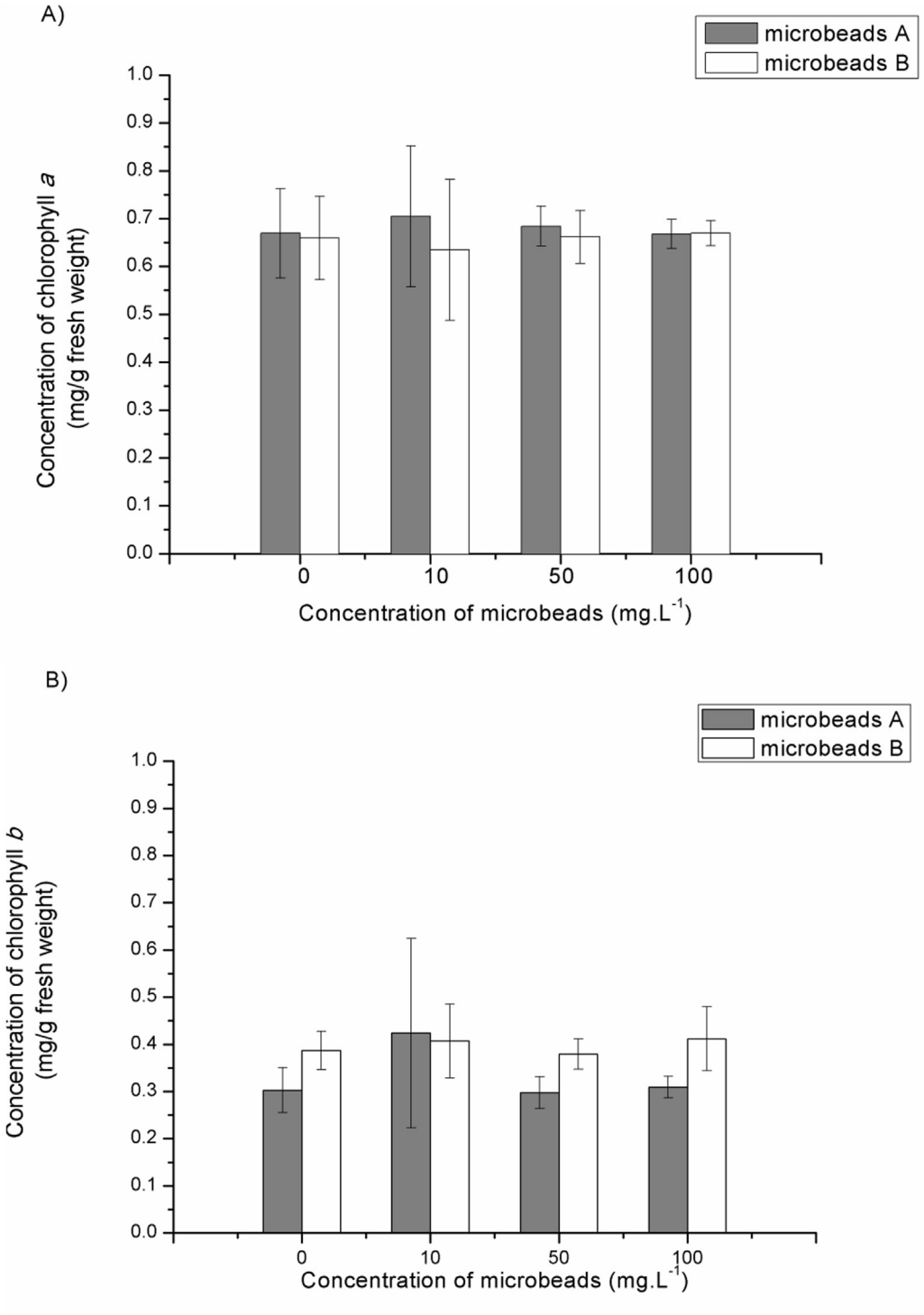

Fig. 2. Chlorophyll *a* (**A**) and chlorophyll *b* (**B**) concentration in the leaves of *Lemna minor* exposed to microbeads A and B. Mean value and standard deviation are shown (n = 6).

product did not considerably affect root cells and roots exposed to 100 mg L^{-1} of microbeads B showed only a very light bluish color comparable to 1 mg L^{-1} of standard compound $K_2Cr_2O_7$.

3.4. Effect of leached cosmetic ingredients on leaves and roots

To evaluate the possible effect of cosmetic ingredients that may leach out from incompletely washed microbeads, an additional leaching experiment was prepared. After seven days of experiment, leachate from the highest concentration of microbeads A and B used in the experiment, 100 mg L^{-1}, caused less than 10% of specific leaf growth rate inhibition in comparison to control (8% and 9%, respectively). The root length was not significantly affected by any of investigated leachates (from the A and B microbeads); the inhibition of root length in leachate from the A and B microbeads in comparison to control was 2% and 3%, respectively (data not

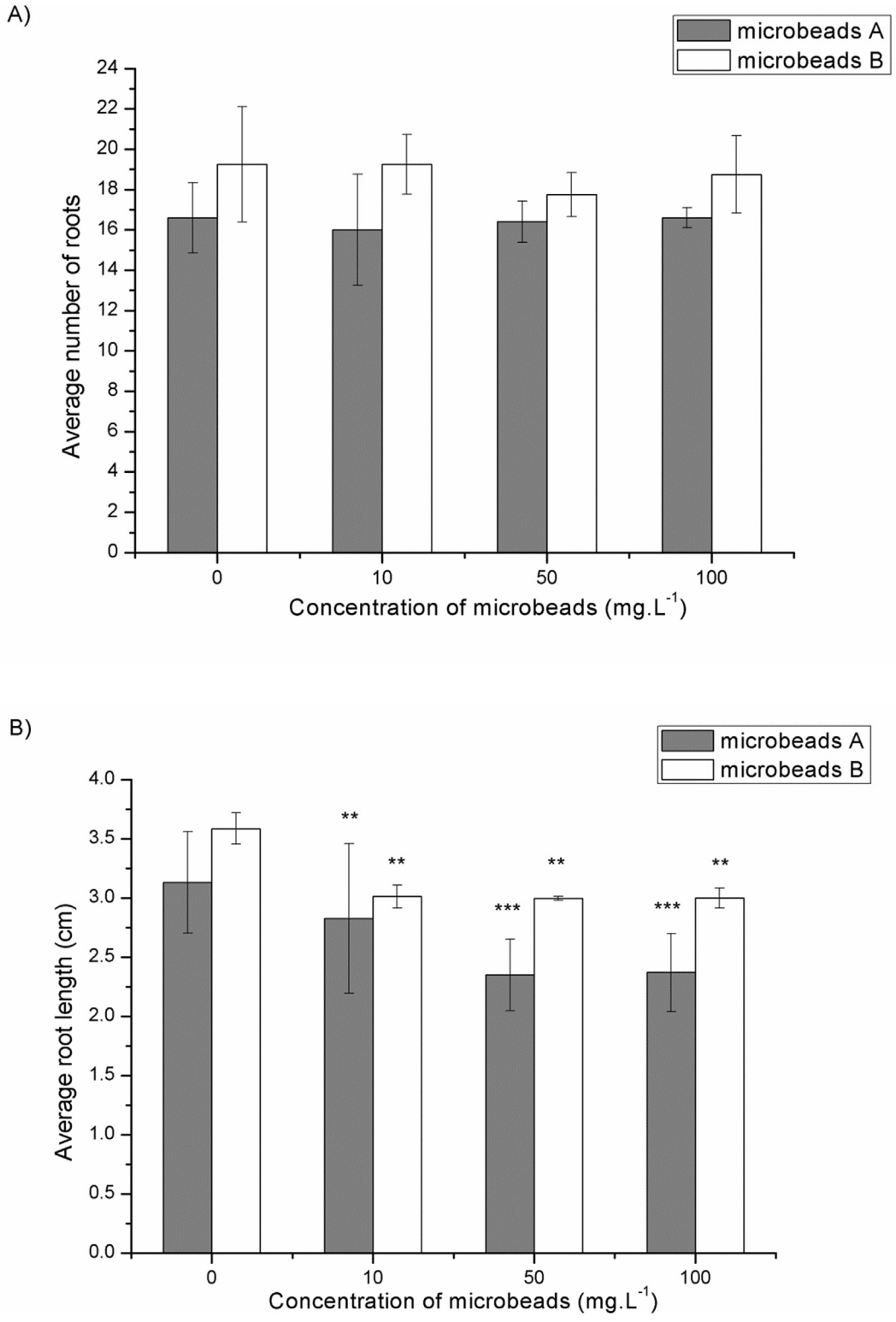

Fig. 3. The effects of microbeads A and B on the root number (**A**) and average root length (**B**) of *Lemna minor*. Mean value and standard deviation are shown (n = 15 for microbeads A and n = 20 for microbeads B). A statistically significant difference in comparison to the control is marked with ** ($0.001<p<0.01$) and *** ($p<0.001$).

shown). Similarly, leachates did not caused any visible staining of roots by Evans blue (Fig. 5). Therefore, the contribution of leached cosmetic ingredients during the experiment can be neglected and the actual impact on duckweed may be attributed only to microbeads.

4. Discussion

In the last decade, polyethylene microbeads have replaced natural exfoliating materials in many cosmetic products (Fendall and Sewell, 2009), which has led to an increase in these particles

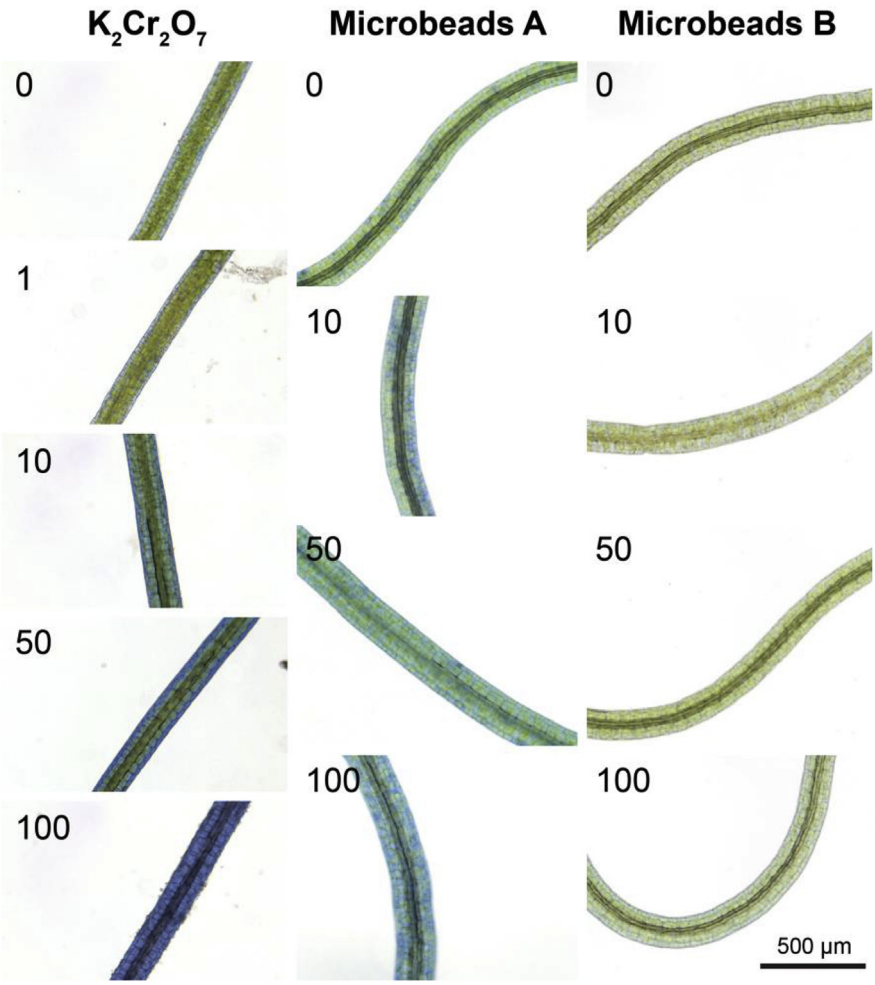

Fig. 4. *Lemna minor* roots stained with 0.05% Evans Blue to visualize dead cells in control (0) and in roots exposed to $K_2Cr_2O_7$ (1, 10, 50, and 100 mg L^{-1}), microbeads A and B (10, 50 and 100 mg L^{-1}).

in the environment (Eerkes-Medrano et al., 2015). Polyethylene microbeads extracted from various cosmetic products usually have irregular shapes and sometimes cosmetic products contain a small portion of smooth spheres. When we compared the photos of microbeads used in our study (purchased in Slovenia) with those obtained from cosmetics purchased in New Zealand (Gregory, 1996; Fendall and Sewell, 2009) or in the USA (Chang, 2015) we found substantially similarly-shaped microbeads. Most of these products have comparable irregularly-shape attributes and can be easily recognized among other MP particles. Nevertheless, microbeads obtained from two different facial body scrubs investigated in this study did differ in their size and shape.

Although there is evidence that polyethylene microbeads from cosmetics are released into freshwaters (Eerkes-Medrano et al., 2015) their environmental concentrations are practically unknown because they have not been separately identified among secondary MP samples. Polyethylene microbeads have a low density between 0.91 and 0.96 g cm^{-1} (Napper et al., 2015), and can thus preferably float on the water's surface and neustonic environment. The concentrations of floating MP (whether polyethylene microbeads from cosmetics or other floating particles) retained in lakes is very variable, e.g. at the remote Lake Khövsgöl, Mongolia the concentration of particles was on average 20,264 particles/km^2 (particles > 333 µm) (Free et al., 2014), in the three of Laurentian Great Lakes, USA it was in average 43,157 particles/km^2 (particles > 355 µm) (Eriksen et al., 2013) and in the Three Gorges Reservoir, China it was 13,617,500 particles/km^2 (particles > 112 µm) (Zhang et al., 2015). However, in the literature, results are often inconsistent due to differences in sampling, identification and enumeration techniques as well as the expression of results (Eerkes-Medrano et al., 2015). Therefore we are unable to estimate whether the microbeads used in this study can be considered environmentally relevant.

Our study showed that ***duckweed leaf growth rate was not significantly affected*** by any concentration of either type of microbeads. The most plausible explanation is that floating polyethylene microbeads leave enough space for normal growth, because it has been shown, that crowding is an important factor in the limitation of duckweed leaf growth (Driever et al., 2005). It is very likely that duckweed overgrows these particles that are then adsorbed on the abaxial part of the leaf. In line with no effect on the leaf growth, we also found no effect of microbeads on the ***amount of photosynthetic pigments*** (chlorophyll *a and b*). Similarly, Sjollema et al. (2016) found no effect of polystyrene MP (0.05, 0.5 and 6 µm) on microalgal photosynthesis. On the contrary, Bhattacharya et al. (2010) observed a reduction in the photosynthetic activity of algal cells when exposed to polystyrene nanoplastics (0.02 µm). They anticipated that MP blocked the access of light to algae, but this was obviously not the case in our study, because microbeads were only under the water and on the abaxial

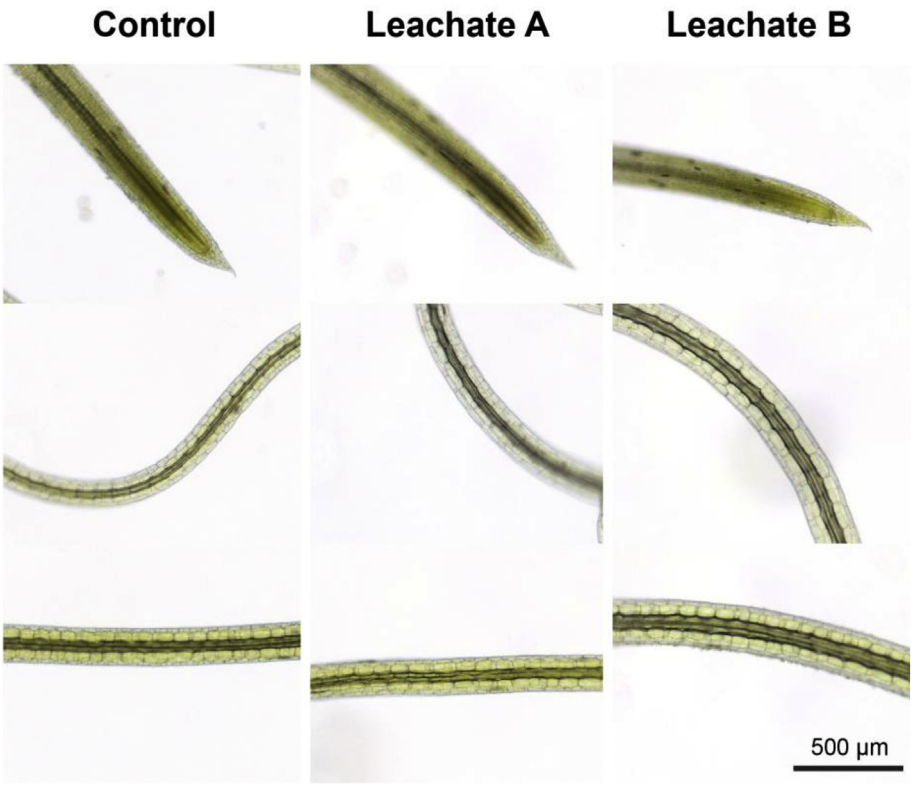

Fig. 5. Roots stained with 0.05% Evans Blue to visualize dead cells in the control and in roots exposed to leachates from microbeads A and B. No visible effects were observed. Three different sections of the root are shown (the tip-upper panel, middle section-middle panel, and the section close to leaves-lower panel).

part of leaves and thus we do not expect that they could block the light source from above. In this study, we exposed the duckweed for 7 days. It remains to be investigated if leaf growth would be affected after a longer exposure period, particularly since the root length was affected.

On the other hand, both microbeads affected the ***root growth of Lemna minor***. We suggest that microbeads were adsorbed onto the surface of the roots and mechanically blocked root growth resulting in reduction of root length. Namely, adsorption of microbead A onto the root surface and mechanical damage of the roots was evidenced as shown in Fig. 6. Bhattacharya et al. (2010) also suggested that positively charged plastic particles are attracted to cellulose constituents of plant cells due to the electrostatic forces and their adsorption is also enhanced by the roughness of the plant cellulose surfaces providing numerous binding sites for plastic particles. Although the charge of MP was not measured, the polyethylene microbeads used in our study expressed a high affinity to negatively charged surfaces, e.g. glass walls.

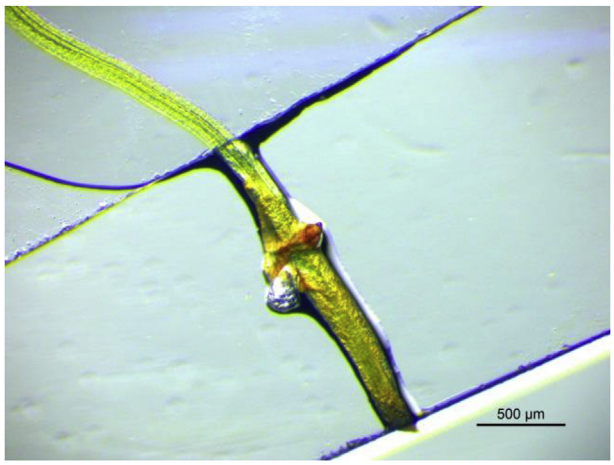

Fig. 6. Adsorption of microbead A onto the root surface.

Root cell viability staining assay showed that A microbeads significantly affected the root cell membrane permeability, while no such effect was found in the case of B microbeads. Both microbeads were of similar size range, but differed in the shape: while A microbeads had sharp edges, B microbeads exhibited a smooth surface (Fig. 1). Therefore, the most plausible explanation is that A microbeads mechanically damage the roots. Interestingly, root length was affected by both types of microbeads, but again A microbeads caused a greater reduction in root length (by approximately 25% in comparison to control) than B microbeads (by approximately 15% in comparison to control). The shape and surface roughness can be a key factor in causing mechanical damage in organisms. For example, Mazurais et al. (2015) observed a harmless and fast transit of smooth polyethylene microbeads through the digestive tract of fish larvae, while Lusher et al. (2013) reported retained plastic debris such as fibers and fragments of various shapes in the guts of wildlife.

Our results showed that leachates from both types of microbeads induced no alterations in all monitored parameters in *Lemna minor*. This is in agreement with our previous study where leachate from PET fibers did not affect daphnids and GC-MS analysis found no chemicals present above the level of detection (Jemec et al., 2016). On the contrary, several researchers have suggested a negative impact caused by leakage of chemical stressors adsorbed on MP (Avio et al., 2015) or additives used during plastics production (Sussarellu et al., 2016). It has also been suggested that in

vertebrates the chemical stressors adsorbed on MP are leached out under acidic gut conditions (Teuten et al., 2009). On the contrary, in invertebrates such as crustaceans, the gut conditions are mostly close to neutral (Hasler, 1935).

We did not observe a clear microbead exposure dose-response relationship in the case of root length and root cell viability. Namely, all concentrations above 10 mg L^{-1} caused a similar level of effect with the exception of the A microbeads effect on root length which was higher in the case of 50 mg L^{-1} compared to 10 mg L^{-1}. Therefore the impact on root length of floating plants above a certain concentration level most probably cannot be clearly linked to the amount of microbeads on the water surface, but more likely is due to its absolute presence or absence. The lack of dose-response is an additional proof that the impact of MP on the plant roots is mechanical and not chemical. In the latter case, it would be expected that the response would depend on the dose.

5. Conclusion

To our knowledge, this is the first report of the impact of polyethylene microbeads on duckweed *Lemna minor*. Our results showed that specific leaf growth rate and content of photosynthetic pigments in duckweed leaves are not negatively affected by polyethylene microbeads. However, investigated particles significantly affected the root growth. Sharp-shaped particles also reduced the viability of root cells. We conclude that microbeads do not present a hazard only to invertebrates and vertebrates after ingestion, but they can have also negative impacts on floating plants in freshwater ecosystems.

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References

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222.

Baldwin, A.K., Corsi, S.R., Mason, S.A., 2016. Plastic debris in 29 Great Lakes tributaries: relations to watershed attributes and hydrology. Environ. Sci. Technol. 50, 10377–10385.

Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ. Sci. Technol. 48, 12336–12343.

Bhattacharya, P., 2016. A review on the impacts of microplastic beads used in cosmetics. Acta Biomed. Sci. 3, 4.

Bhattacharya, P., Lin, S., Turner, J.P., Ke, P.C., 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J. Phys. Chem. C 114, 16556–16561.

Bouwmeester, H., Hollman, P.C.H., Peters, R.J.B., 2015. Potential health impact of environmentally released micro- and nanoplastics in the human food production chain: experiences from nanotoxicology. Environ. Sci. Technol. 49, 8932–8947.

Carr, S.A., Liu, J., Tesoro, A.G., 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. 91, 174–182.

Chang, M., 2015. Reducing microplastics from facial exfoliating cleansers in wastewater through treatment versus consumer product decisions. Mar. Pollut. Bull. 101, 330–333.

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597.

Driever, S.M., Nes, E.H.v., Roijackers, R.M.M., 2005. Growth limitation of *Lemna minor* due to high plant density. Aquat. Bot. 81, 245–251.

Eerkes-Medrano, D., Thompson, R.C., Aldridge, D.C., 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. 75, 63–82.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., Amato, S., 2013. Microplastic pollution in the surface waters of the laurentian Great lakes. Mar. Pollut. Bull. 77, 177–182.

Fendall, L.S., Sewell, M.A., 2009. Contributing to marine pollution by washing your face: microplastics in facial cleansers. Mar. Pollut. Bull. 58, 1225–1228.

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. 85, 156–163.

Gregory, M.R., 1996. Plastic 'scrubbers' in hand cleansers: a further (and minor) source for marine pollution identified. Mar. Pollut. Bull. 32, 867–871.

Hasler, A.D., 1935. The physiology of digestion of plankton crustacea: I. Some digestive enzymes of Daphnia. Biol. Bull. 68, 207–214.

Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., Laforsch, C., 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. Curr. Biol. 23, R867–R868.

ISO, 2005. ISO 20079, Determination of the Toxic Effect of Water Constituents and Waste Water on Duckweed (*Lemna Minor*) - Duckweed Growth Inhibition Test. Geneva.

Jemec, A., Horvat, P., Kunej, U., Bele, M., Kržan, A., 2016. Uptake and effects of microplastic textile fibers on freshwater crustacean Daphnia magna. Environ. Pollut. 219, 201–209.

Kalčikova, G., Žgajnar Gotvajn, A., 2016. Quantification of microplastics emissions from cosmetic products to freshwater ecosystems. In: SETAC Europe 26th Annual Meeting: 22-26 May 2016, Nantes, France.

Lichtenthaler, K.H., 1987. Chlorophylls and carotenoids—pigments of photosynthetic biomembranes. Methods Enzym. 148, 350–382.

Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99.

Mattsson, K., Ekvall, M.T., Hansson, L.-A., Linse, S., Malmendal, A., Cedervall, T., 2015. Altered behavior, physiology, and metabolism in fish exposed to polystyrene nanoparticles. Environ. Sci. Technol. 49, 553–561.

Mazurais, D., Ernande, B., Quazuguel, P., Severe, A., Huelvan, C., Madec, L., Mouchel, O., Soudant, P., Robbens, J., Huvet, A., Zambonino-Infante, J., 2015. Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (*Dicentrarchus labrax*) larvae. Mar. Environ. Res. 112, 78–85.

Mintenig, S.M., Int-Veen, I., Löder, M.G.J., Primpke, S., Gerdts, G., 2017. Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging. Water Res. 108, 365–372.

Napper, I.E., Bakir, A., Rowland, S.J., Thompson, R.C., 2015. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. Mar. Pollut. Bull. 99, 178–185.

Prokin, A.A., Dubov, P.G., Bolotov, S.E., 2015. Formation of macroinvertebrates communities in duckweed (Lemnaceae) and artificial surface-floating substrate: results of the experiment under natural conditions. Inland Water Biol. 8, 373–383.

Radić, S., Pevalek-Kozlina, B., 2010. Effects of osmotic stress on antioxidative system of duckweed (*Lemna minor* L). Period. Biol. 112, 293–299.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat. Meth 9, 676–682.

SFRA0025, 2015. Identification and Assessment of Riverine Input of (Marine) Litter. Final report for the European Commission DG Environment under Framework Contract No. ENV.D.2/FRA/2012/0025.

Sjollema, S.B., Redondo-Hasselerharm, P., Leslie, H.A., Kraak, M.H.S., Vethaak, A.D., 2016. Do plastic particles affect microalgal photosynthesis and growth? Aquat. Toxicol. 170, 259–261.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. 113, 2430–2435.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Phil. Trans. R. Soc. B Biol. Sci. 364, 2027.

Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304, 838–838.

Van Hoeck, A., Horemans, N., Monsieurs, P., Cao, H.X., Vandenhove, H., Blust, R., 2015. The first draft genome of the aquatic model plant *Lemna minor* opens the route for future stress physiology research and biotechnological applications. Biotechnol. Biofuels 8, 188.

Zhang, K., Gong, W., Lv, J., Xiong, X., Wu, C., 2015. Accumulation of floating microplastics behind the three Gorges dam. Environ. Pollut. 204, 117–123.