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MOURA, D.S., PESTANA, C.J., MOFFAT, C.F., HUI, J., IRVINE, J.T.S., EDWARDS, C. and LAWTON, L.A.

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Adsorption of cyanotoxins on polypropylene and polyethylene terephthalate: Microplastics as vector of eight microcystin analogues☆

Diana S. Moura [a,*], Carlos J. Pestana [a], Colin F. Moffat [a], Jianing Hui [b], John T.S. Irvine [b], Christine Edwards [a], Linda A. Lawton [a]

[a] School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, AB10 7GJ, UK
[b] School of Chemistry, University of St Andrews, North Haugh, St Andrews, Scotland, KY16 9ST, UK

ABSTRACT

Plastics are utilised globally but are of environmental concern due to their persistence. The global presence of microplastics (particles <5 mm in all dimensions) in freshwater environments is increasingly reported, as has the presence of cyanobacterial toxins, including the microcystins. We elucidated the potential role of microplastics as a vector for eight microcystin analogues. Two sizes of polypropylene (PP) and polyethylene terephthalate (PET) microparticles were evaluated. The median particle size distribution (D_{50}) was 8–28 μm for small particles, and 81–124 μm for large particles. Additionally, microcystin-LR and -LF were evaluated individually using small PP and PET to elucidate the adsorption behaviour in the absence of competition. Microcystin hydrophobicity, polymer material, and particle size were key factors influencing adsorption to the plastic microparticles. The small size PP microparticles demonstrated a high affinity for the 8 microcystin analogues. The proportion of microcystin adsorbed onto the small particles of PP after 48 h contact was between 83 and 100%, depending on the analogue. Of all analogues investigated, only microcystin-LW and -LF adsorbed onto the larger sized PP and PET microparticles. Individually, greater amounts of MC-LF adsorbed onto the small PET (19%) compared to when it was present in the mixture of microcystins (11%). While MC-LR did not adsorb onto small PET microparticles in the mixture, 5% adsorption was observed when individually in contact with small PET microparticles. The results demonstrated that microplastics can adsorb eight different microcystin analogues and that more hydrophobic analogues are more likely to adsorb than less hydrophobic analogues.

1. Introduction

Plastics are of global environmental concern due to their persistence and potential to harm wildlife. However, plastics will continue to occupy a highly relevant place in contemporary society, due to their low cost, lightweight and durable properties (Sorensen and Jovanović, 2021; Thompson et al., 2009). World plastic production reached almost 370 million tonnes in 2019 (Plastics Europe, 2020), a large proportion of which is used for single-use packaging (Dris et al., 2015), which can represent 10–15%, by weight of municipal solid waste (Andrady, 2017). Plastic waste can enter the environment from poorly managed landfill sights or carelessly discarded post-consumer products (Dris et al., 2015). Plastic fragments covering a wide range of sizes can enter aquatic systems (Napper and Thompson, 2019), with particles smaller than 5 mm (in all dimensions) defined as microplastic. These microparticles are divided into primary (small particle plastics that are manufactured at that size) and secondary (plastic fragments derived from larger pieces of plastic by physical, chemical and biological interaction, Thompson et al., 2009). The most commonly reported microplastics in the environment are secondary microplastics (Weithmann et al., 2018) and their detection has increased globally according to recent reports (Li et al., 2018a). For aquatic environments, the majority of studies have focussed on marine systems, with less than 4% of studies focussed on freshwater environments (Li et al., 2018a; Vaughan et al., 2017). Untreated wastewater (e.g. from combined sewage overflow) and wastewater treatment plant effluents are one of the main sources of microplastic in the freshwater environment (Anderson et al., 2016). Additionally, wastewater also contributes significantly to the input of nutrients into the aquatic environment, which can lead to mass occurrences of cyanobacteria known as blooms. Some cyanobacterial strains can produce

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harmful secondary metabolites often referred to as cyanotoxins, the most commonly reported cyanotoxins are the group of the microcystins. Microcystin presence in water bodies has caused illness and death of wild and domestic animals around the world (Sivonen and Jones, 1999). Microplastics have been shown to enter the food web (D'Souza et al., 2020), which can impact wildlife with recent reports indicating that microplastics have also been ingested by humans (Li et al., 2018b; Orb, 2017). Polypropylene (PP) and polyethylene terephthalate (PET) were found in the faeces of all human participants in a recent study carried out in Vienna, Austria, on the effect of plastic food packaging (Liebmann et al., 2018). The presence of microplastics in the food web is of concern, especially, based on the fact that microplastics have been shown to act as vectors for hydrophobic organic pollutants in aquatic systems (Yu et al., 2019). The interaction of two individual microcystins (MC-LR or MC-LF) with microparticles of PET, polyethylene (PE), polyvinyl chloride (PVC), and polystyrene (PS) has been explored at five different pHs where the hydrophobic analogue microcystin-LF showed great adsorption on the smallest size (90–125 μm) of PS at pH 7 (Pestana et al., 2021). Pestana et al. (2021) have identified microcystin hydrophobicity, microplastic particle size, and polymer type as the main drivers of microcystin-microplastic interaction. Thus, the current study set out to determine the microcystin-microplastic interactions of smaller particle sizes (D_{50} 8–28 μm), an additional microplastic type (PP), and six additional microcystin analogues with a wide range hydrophobicities. Further, the potential of competition for binding sites of a mixture of eight microcystin analogues in contact with two different sizes of PP and PET (both are widely reported in freshwater systems; Koelmans et al., 2019) was elucidated. The potential competition behaviour of microcystin analogues for adsorption binding sites was evaluated by placing small particles of PP and PET in contact with two microcystin analogues individually and comparing the data from this experiment with data from when the two microcystins are present in a mixture.

2. Materials and methods

2.1. Microplastics and chemicals

Polyethylene terephthalate (PET) 15 μm (small) and 100 μm (large) along with polypropylene (PP) 25 μm (small) and 100 μm (large) were acquired from Shanghai Guanbu Electromechanical Technology Co. Ltd., China and used as received. Artificial freshwater (AFW) was used as the experimental medium. AFW was prepared with ultrapure water (18.2 MΩ) and the addition of $CaCl_2.2H_2O$ (58.5 mg L^{-1}), $MgSO_4.7H_2O$ (24.7 mg L^{-1}), $NaHCO_3$ (12.0 mg L^{-1}), and KCl (1.2 mg L^{-1}) according to Akkanen and Kukkonen (2003). The pH was adjusted to 7 either with HNO_3 or NaOH. Chemicals used were acquired from Fisher Scientific UK Ltd (UK) at analytical grade. Microcystin analogues were acquired as per Enzo Life Sciences, >95% purity. MC-RR, -YR, -LR, -WR, -LA, -LY, -LW, and -LF, where the letters after the hyphen describe the variable amino acids (figure S1 for key to the amino acid letters), from the least to the most hydrophobic analogue, were selected due to their different hydrophobicities (figure S1). The hydrophobicity order of the analogues was determined according to the liquid chromatographic separation of the microcystins (figure S2).

2.2. Characterisation of physical properties of microplastics

A Nicolet iS10 FT-IR Spectrometer from Thermo Fisher Scientific (UK) with OMNIC Spectra Software was used to analyse the microplastics. The FT-IR scanning wavenumber was set from 400 to 4000 cm^{-1}. Samples were scanned 32 times, the resolution was set at 8 cm^{-1}. No correction was applied. The BET surface area (S_{BET}) was determined on a Tristar II surface area and porosity instrument (Micromeritics, UK). Before the test, the microplastics were dried under vacuum using a VacPrep degasser (Micromeritics, UK) at 30 °C for 24 h. This removed surface adsorbed gas and moisture. Particle size analysis (PSA) was

carried out on a Mastersizer 2000 particle size analyzer (Malvern Pan-alytical, UK). Furthermore, a simulated surface area (S_{PSA}) was calculated by the PSA software modelling the particles as perfect spheres and not accounting for the porosity and roughness of the material. Scanning electron microscopy (SEM, Scios DualBeam, Thermo Fisher Scientific, UK) was employed to investigate the morphologies of the microplastics. For crystallinity composition evaluation, powder X-ray diffraction (XRD) was carried out on an Empyrean diffractometer (Malvern Pan-alytical, UK) in reflection mode with a primary beam monochromator (Cu Kα1).

See the Supplementary Material for detailed FT-IT spectra (figure S4), particle size distribution (figure S5), and XRD patterns (figure S6).

2.3. Determination of microcystin analogue concentration

Analysis of the microcystins was performed using high performance liquid chromatography (HPLC; Waters Corporation, UK). The equipment included a solvent delivery system (Alliance 2695) with detection by photodiode array (PDA, Alliance 2996). The PDA scanning wavelength was set from 200 to 400 nm. Separation of microcystin analogues was achieved using a Symmetry dC18 column (2.1 mm internal diameter x 150 mm; 5 μm particles size) which was maintained at 40 °C. The mobile phases were ultra-pure water (18.2 MΩ) (A) and acetonitrile (B) each containing 0.05% (v/v) trifluoroacetic acid (TFA; Fisher Scientific Ltd, UK). The flow rate was 0.3 mL min^{-1}. A linear gradient was used for the separation of the microcystin analogues (table S1). The separation of MC-RR, -YR, -LR, -WR, -LA, -LY, -LW, and -LF followed this order, which is directly related to their hydrophobicity (figure S2). The limit of quantification of the microcystin analogues using this method was 0.05 μg mL^{-1}.

2.4. Adsorption of microcystin analogues on microplastics

A solution containing a mixture of eight microcystin analogues (microcystin-RR, -YR, -LR, -WR, -LA, -LY, -LW and -LF each at 5 μg mL^{-1} (4.96 ± 0.26 μg mL^{-1}, n = 24) as well as a solution containing either MC-LF (4.65 ± 0.19 μg mL^{-1}, n = 3) or MC-LR (4.60 ± 0.02 μg mL^{-1}, n = 3) in AFW were prepared. All samples were removed using a 100 μL glass syringe with a stainless-steel needle (Hamilton, UK) to avoid contact of the toxin solution with laboratory plastics. MC solutions (2.5 mL) were combined with plastics particles (25 mg equivalent to 10 g L^{-1}) in glass vials (4 mL). The mixture of the eight microcystin analogues was tested with PET and PP at each of the two particle sizes. Additionally, solutions of either MC-LR or -LF were placed in contact individually with the small particles of PP and PET to investigate the impact on the amount of microcystin adsorbed on microplastics when the compounds are present individually. A comparison was then made with the data from when multiple analogues were present to determine if there is binding competition.

Samples were continuously horizontally agitated on a MaxQ 6000 orbital shaker (Thermo Scientific, UK) at 200 rpm and 25 °C in the dark for 48 h. Samples (200 μL) were taken at 2, 4, 6, 8, 12, 24, and 48 h and filtered using a microcentrifuge tube filter (2 mL spin-X tubes made of PP, cellulose acetate filter, 0.45 μm pore size, Corning USA). Samples (100 μL) were then analysed by HPLC-PDA. A control containing either the eight microcystin analogues, MC-LR or -LF without microplastic particle was also prepared and analysed at each sampling point. All experiments and controls were conducted in triplicate. Throughout the investigation, contact with laboratory plastics was eliminated except for the filtration device which could not be avoided. Controls indicated that the loss through this step was between 4 ± 1% (MC-LR) to 11 ± 1% (MC-LW). The adsorption was calculated by the difference on MC concentration in the samples with microplastics and in the control.

2.5. Statistical analysis

The amount of microcystin adsorbed per unit mass of microplastics ($\mu g\ g^{-1}$), was estimated using equation (1):

$$q_{(t)} = \left(C_{ctrl(t)} - C_{(t)}\right)V \Big/ m \tag{1}$$

where.

- $q_{(t)}$ is the amount of microcystin adsorbed on microplastics ($\mu g\ g^{-1}$) at sampling time t
- $C_{ctrl(t)}$ is the control solution concentration of microcystin ($\mu g\ mL^{-1}$) at the sampling time t as determined by HPLC-PDA
- $C_{(t)}$ is the sample solution concentration of microcystin ($\mu g\ mL^{-1}$) at the sampling time t as determined by HPLC-PDA
- m is the mass of plastic added to the vial (in g)
- V is the total volume of solution (in mL) in the vial

The percentage of microcystin adsorbed onto the microplastic was calculated using equation (2):

$$\%Adsorbed_{(t)} = \left(\left(C_{ctrl(t)} - C_{(t)}\right)x100\right)\Big/ C_{ctrl(t)} \tag{2}$$

where.

- $\%Adsorbed_{(t)}$ is the percent of microcystin adsorbed on microplastics at sampling time t
- $C_{ctrl(t)}$ is the control solution concentration of microcystin ($\mu g\ mL^{-1}$) at the sampling time t as determined by HPLC-PDA
- $C_{(t)}$ is the sample solution concentration of microcystin ($\mu g\ mL^{-1}$) at the sampling time t as determined by HPLC-PDA

The Shapiro-Wilk normality test was applied to evaluate the distribution of the data. The test returned that normal distribution of the data cannot be assumed. Consequently, a Wilcoxon rank sum test was carried out to perform significance testing (Supplemental Information S1.4). For all statistical tests, a significance level of 5 percent was set.

3. Results and discussion

3.1. Microplastics characterisation

The microplastic particles were characterised to confirm the specifications provided by the manufacturer. FT-IR analysis of the microplastics showed that the spectra of the particles analysed matched the corresponding spectra for PET and PP in the instrument database (figure S4). The overall size of the microplastics, however, did not match with the sizes provided by the manufacturer. For the smallest size range, the median size (D_{50}) of the PP particles was 8 μm, and 28 μm for PET (figure S5, Fig. 1), instead of 25 μm and 15 μm, respectively. For the large particles (100 μm according to the supplier), PP was determined to have a D_{50} of 124 μm and PET a D_{50} of 81 μm. Furthermore, both sizes of PET showed a wide size distribution (figure S5), for instance, the large PET showed particles from 4 up to 240 μm (Table 1).

The microplastics in the current study had varied particle shapes (Fig. 1). Both microplastic types were irregularly shaped (fragments), representative of what is commonly encountered in the environment (Meng et al., 2019; Koelmans et al., 2019). SEM images confirm the size of the particles determined by the PSA (figure S5). Both sizes of PET give a wide range of sizes (small PET 7–91 μm, large 4–240 μm, Table 1; Fig. 1). Large PET appears to have small plastic debris not present in large PP, which is consistent with the PSA (Fig. 1).

PSA can provide simulated surface area (S_{PSA}) however it assumes perfect sphericity and does not account for porosity, BET analysis (S_{BET}), on the other hand, allows determination of the surface area taking account of roughness and porosity. Small PP particles show a very high surface area 52.2 $m^2\ g^{-1}$ compared to the other microplastics in the current study (Table 1). All simulated surface areas (S_{PSA}) were smaller than those determined by BET (S_{BET}), suggesting a degree of porosity and/or roughness for the microplastic particles. PP showed greater S_{BET} than PET. Potentially because rubbery polymers, like PP, have a relatively expanded, flexible structure, whereas glassy polymers, like PET, have a more rigid, condensed structure (Guo et al., 2012; Xing et al., 1996).

Microplastic crystallinity was evaluated by XRD analysis. Polymers are classified into crystalline, semi-crystalline, and amorphous states according to the degree of regularity of their molecular chain arrangement (Guo et al., 2012). PET and PP are known as semi-crystalline materials which was confirmed from their XRD patterns (figure S6).
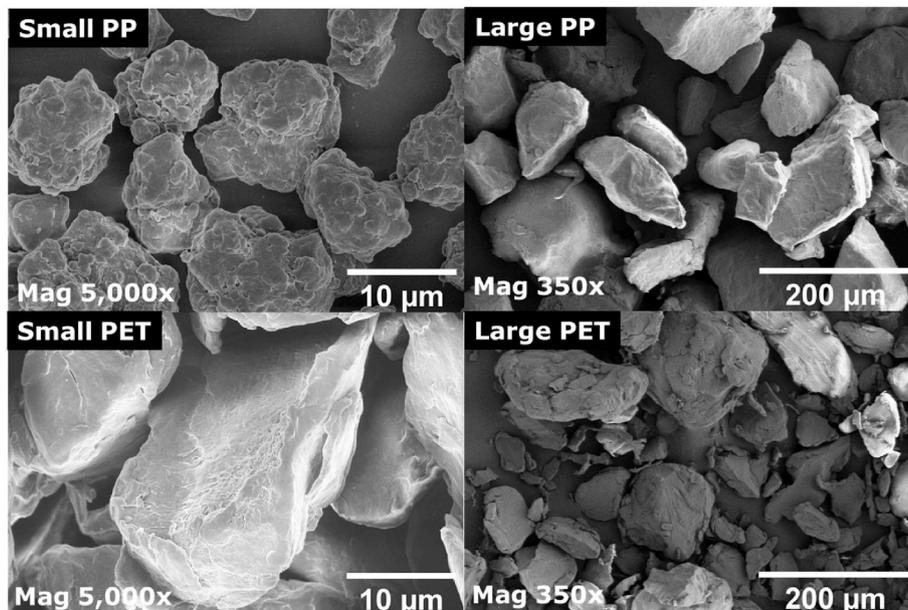
Fig. 1. Scanning electron microscopic images of polypropylene (PP) and polyethylene terephthalate (PET) microplastics used in this study.

Table 1

Microplastic characterisation: Plastic polarity, glass transition temperature (Tg), supplier specified particle size, particle size analysis (PSA) range, PSA median of the particle size distribution (D_{50}), simulated surface area (S_{PSA}), BET surface area (S_{BET}) and XRD intensity peaks.

Plastic	Plastic polarity	Tg	Particle size -supplier	PSA range	PSA D_{50}	S_{PSA}	S_{BET}	XRD intensity peaks
	–	°C	μm	μm	μm	$m^2 g^{-1}$	$m^2 g^{-1}$	counts
PP	Non-polar[a]	0	25 (Small)	4–23	8	0.72	52.2	22,633
			100 (Large)	60–275	124	0.05	0.73	20,366
PET	Polar[b]	69	15 (Small)	7–91	28	0.31	0.82	6,727
			100 (Large)	4–240	81	0.10	0.46	7,487

[a] Li et al. (2018c) and.

[b] Seki et al. (2014).

Crystalline regions are well organised structures represented by the presence of peaks in XRD patterns. Amorphous regions are represented by the absence of sharp peaks in XRD patterns. They are flexible, randomly orientated polymer chains (Atugoda et al., 2021) that can enhance the adsorption of compounds. The crystallinity of the polymer is directly proportional to the diffraction peak intensity of the XRD (Li et al., 2019). PP has a higher degree of crystallinity demonstrated by a peak intensity of ~20,000 with PET showing a crystallinity peak intensity of ~7000 (figure S6, Table 1). As would be expected, the XRD analysis did not show a difference in the crystallinity between the sizes
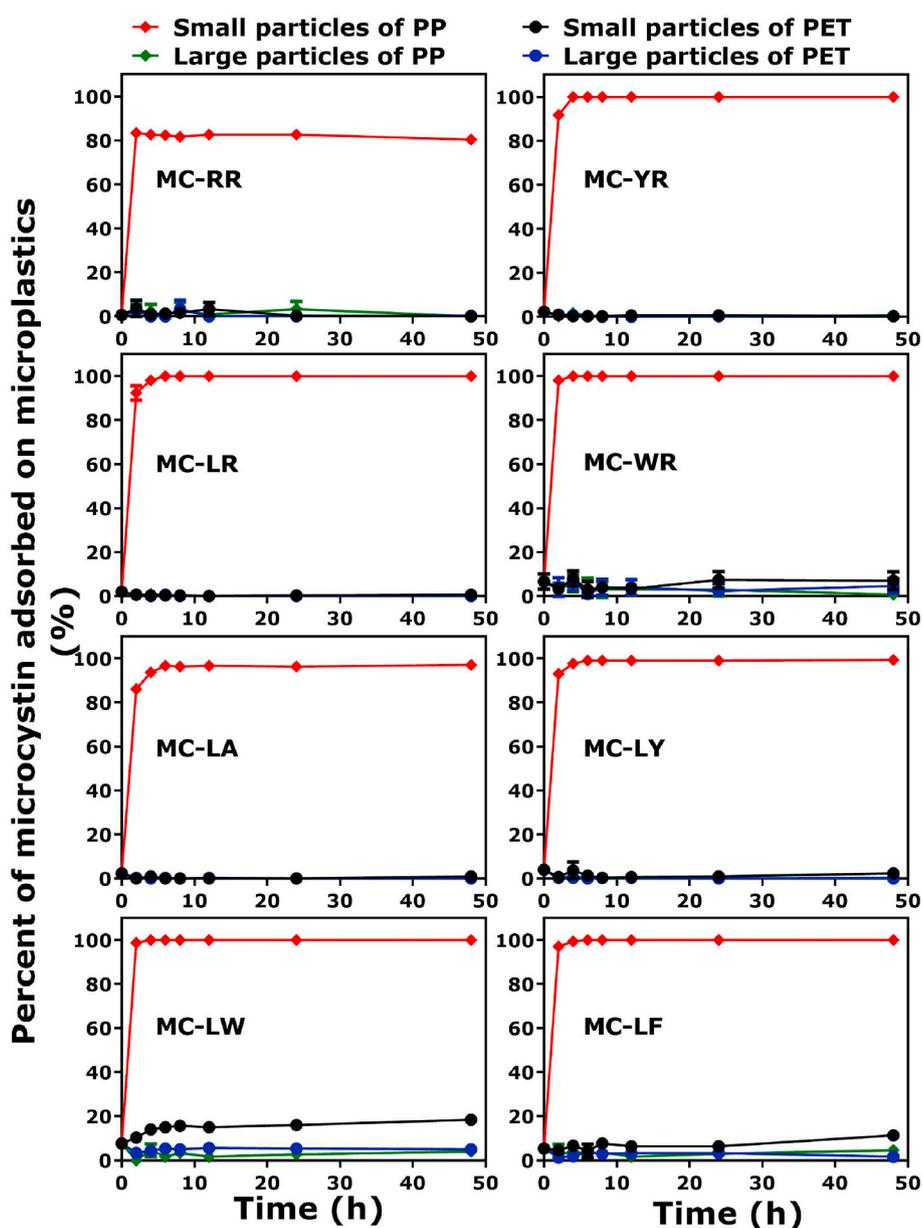
Fig. 2. Percent of microcystin (MC) analogues adsorbed on polypropylene (PP) and polyethylene terephthalate (PET) small (D_{50} 8–28 μm) and large (D_{50} 81–124 μm) microplastic particles sizes over 48 h in artificial freshwater with horizontal agitation in the dark. The percent adsorbed was estimated using equation (2). The control consisted of a mixture of the eight microcystin analogues without microplastics. n = 3, errors bars = 1 SD.

of the microplastics analysed.

3.2. Effect of size of the microplastic on adsorption of microcystin

The mixture of eight different microcystin analogues was tested in contact with PP and PET at sizes widely reported in the freshwater environment (Koelmans et al., 2016). The highest adsorption of microcystins occurred with the smallest size of microplastics. Five of the eight microcystin analogues in the mixture (except MC-RR, -LA, and -LY) were undetected in solution after 4 h of contact with small PP, which indicates 100% apparent adsorption for MC-YR, -LR, -WR, -LW and -LF (Fig. 2). This is equivalent to approximately 500 μg of each microcystin analogue adsorbed per gram of PP (Fig. 2). After 4 h contact, MC-RR, -LA, and -LY had adsorbed onto the small PP by between 83 (MC-RR) and 94% (MC-LA and -LY). For the large PP, however, only the most hydrophobic microcystin analogues (MC-LW and MC-LF) were adsorbed. Furthermore, the amount adsorbed was relatively low (approximately $4 \pm 1\%$ for both MC-LW and -LF, Fig. 2). Considering all analogues, the total amount of microcystin adsorbed on small PP was $\sum$MC 3,529 μg g^{-1} in contrast to $\sum$MC 52 μg g^{-1} (Fig. 3) on large PP. Again, greater amounts adsorbed on the smaller particles of PET ($\sum$MC 181 μg g^{-1}) compared to large PET ($\sum$MC 50 μg g^{-1}, Fig. 3).

In addition to the hydrophobic analogues (MC-LW and -LF), MC-WR also showed adsorption on small and large PET particles. As observed by our previous study (Pestana et al., 2021), with particle size increase, the amount of microcystin adsorbed onto microplastics decrease. Zhan et al.

(2016) and Wang et al. (2019) also demonstrated that the amount of a compound that is adsorbed is directly related to the adsorbent particle size. Zhan et al. (2016) found a threefold adsorption of a polychlorinated biphenyl (PCB77) on the small PP particles (180–425 μm) in contrast to the larger range investigated (425–850, 850–2,000, and 2,000–5000 μm) with similar observations made by Wang et al. (2019) investigating the adsorption of cadmium onto high-density polyethylene.

3.3. Effect of the type of microplastic on adsorption of microcystin

The polymer type was demonstrated to affect microcystin adsorption. Small PP demonstrated the greatest adsorption of microcystins ($\sum$MC 3,529 μg g^{-1}) compared to small PET ($\sum$MC 181 μg g^{-1}, Fig. 3). Kamp et al. (2016) also demonstrated greater adsorption of microcystin analogues (MC-LR, -LA, and -LF) onto containers made of PP (approximately 40–80% adsorption after 48 h contact) compared to the other materials investigated (polyethylene terephthalate glycol, polystyrene, high density polyethylene, and polycarbonate).

Alimi et al. (2018), concluded that PP has a greater adsorption capacity when compared with PET. The different adsorption behaviour can be explained by their distinct properties such as glassiness, polarity, and crystallinity. The glassiness of a polymer is measured by the glass transition temperature (Tg; Endo and Koelmans, 2019). In general, polymers with a Tg below ambient temperature (25 °C), are considered rubbery, whereas polymers with a Tg above ambient temperature are glassy polymers (Alimi et al., 2018). Rubbery polymers such as PP (Tg: 0 °C) are often soft and flexible and are expected to allow greater diffusion of contaminants onto and into the polymer than glassy polymers such as PET (Tg: 69 °C, Table 1, Misumi, 2011; Pascall et al., 2005). The polymer chains in the rubbery state have a higher mobility that allows easier penetration of adsorbate (Grinsted et al., 1992). This characteristic can also explain the greater surface area of PP compared to PET demonstrated by the BET analysis (Table 1). In the current study the higher adsorption potential of rubbery polymers was confirmed for small particles with PP having a greater adsorption for microcystins than PET. However, the glassiness of the microplastics is not the only aspect affecting the adsorption of compounds. The antibiotics sulfamethazine (log K_{OW} 0.14) and sulfamethoxazole (log K_{OW} 0.89) showed greater adsorption on the glassy polymer, polyamide (Tg: 47 °C) than on the rubbery polymer, polyethylene (Tg: −125 °C; Guo et al., 2019a; 2019b). The polarity of polyamide (polar) compared to polyethylene (non-polar) was found to be the driving force for adsorption of the hydrophilic antibiotics.

The degree of crystallinity of the microplastics can also affect the adsorption of microcystin. Normally, the higher the degree of crystallinity, the lower the permeability of the polymer. Pestana et al. (2021) showed that the amorphous polymer, PS, showed greater adsorption than glassy polymers such as PET for microcystins-LR and -LF. The high crystallinity peaks for PP (figure S6, Table 1) suggests that PP should have a lower adsorption potential than PET, however the opposite was observed. Li et al. (2018c) also demonstrated no correlation between the crystallinity and the adsorption of antibiotics on microplastics, where the semi-crystalline polymer polyamide showed greater adsorption than amorphous polymers, such as polystyrene, and polyvinyl chloride. These observations suggest that although the crystallinity of the microplastics plays a role in the adsorption by microplastics, it is not one of the main drivers of the interaction.

Results indicate that the main adsorption mechanism taking place on small PP is pore-filling due to the high surface area of small PP. Usually, pore-filling is the interaction responsible for the adsorption of glassy polymers (e.g., PET), however, recently rubbery polymers (e.g., PP) were observed to display a combination of partition and adsorption (pore-filling) during the sorption on plastics (Atugoda et al., 2021; Uber et al., 2019). For large PP and both sizes of PET, hydrophobic interactions appear to be the main factor influencing the adsorption of microcystin on microplastics. No adsorption of hydrophilic analogues
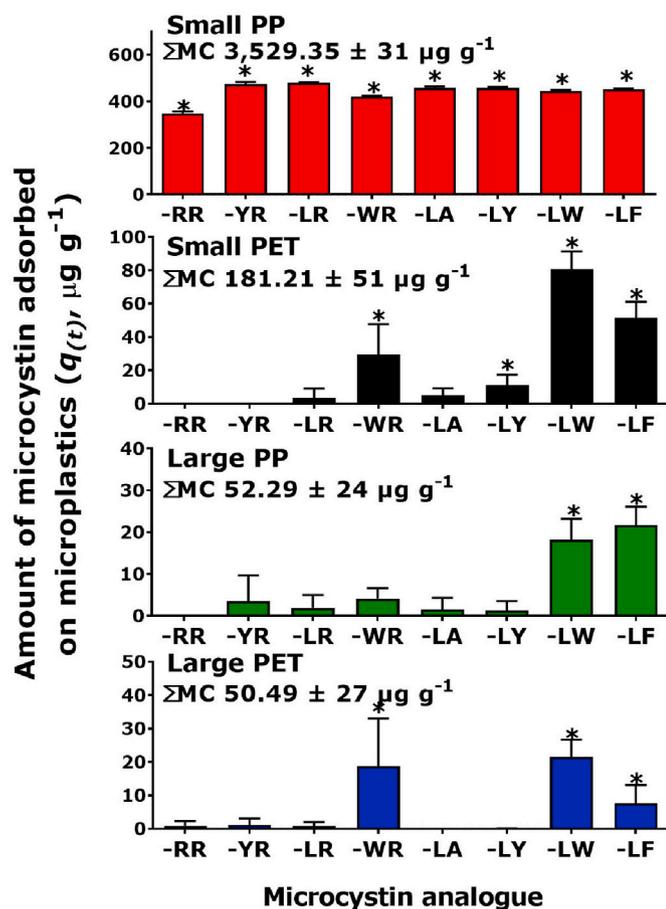
Fig. 3. Amount of microcystin adsorbed onto small (D$_{50}$ 8–28 μm) and large (D$_{50}$ 81–124 μm) polypropylene (PP) and polyethylene terephthalate (PET) per microcystin analogue after 48 h in a mixture of the microcystins. n = 3, errors bars = 1 SD. The amount adsorbed (qt) was estimated using equation (1). The control consisted of a mixture of the eight microcystin analogues without microplastics. * significant difference from the control, $p < 0.05$.

was observed on large PP, and PET, when in a mixture.

According to Guo et al. (2019b), the polarity of microplastics could influence the adsorption mechanisms, which was corroborated in the current study, where the hydrophilic MC-WR (figure S1) adsorbed onto the polar PET (Seki et al., 2014) but not onto the non-polar large PP particles (Fig. 3).

3.4. Effect of microcystin analogue hydrophobicity on adsorption

The more hydrophobic microcystin analogues appear to have higher affinity to both microplastics compared to the more hydrophilic analogues (Fig. 3). This suggests that the chemical structure of the microcystin has a greater effect on the adsorption behaviour on microplastics than properties of the microplastics such as their crystallinity. The eight microcystin analogues studied adsorbed onto small PP after 48 h of contact, while only four analogues (MC-LF, - LW, -LY and -WR) adsorbed in smaller amounts onto PET of the same size range. On small PET, the highest amount of adsorption was recorded for MC-LW and MC-LF with $18 \pm 2\%$ and $11 \pm 2\%$ respectively, followed by $7 \pm 4\%$ adsorption of MC-WR ($p < 0.0005$, Fig. 2). Even though the adsorption of MC-LY onto small PET is significantly different from the control ($p < 0.0009$), its adsorption represented only 2% of the total available microcystin when compared to the control. While a similar total amount of microcystin adsorbed on PP and PET, differences in the composition of the adsorbed microcystin analogues was observed. A greater amount of MC-LF, the most hydrophobic microcystin of the mixture, adsorbed onto large PP ($5 \pm 1\%$) than on large PET ($2 \pm 1\%$, Fig. 2). On the other hand, MC-WR showed $4 \pm 3\%$ adsorption on large PET, while no adsorption of MC-WR was detected for large PP.

The nature of the variable amino acids can substantially influence the overall hydrophobicity of the microcystin molecule (De Maagd et al., 1999). For instance, MC-LF contains the nonpolar amino acids leucine (L) and phenylalanine (F) in positions X and Z respectively (figure S1) making it the most hydrophobic analogue among the microcystins analysed. On the other hand, because of the presence of the polar amino acid arginine (R) in both variable positions (X and Z) MC-RR is the least hydrophobic microcystin in the current study. Besides the polarity, the presence of aromatic rings and the size of the amino acid chain can influence the hydrophobicity of the microcystin (figure S1). Small amino acids present stronger hydrophobic behaviour caused by their smaller volume and larger surface-to-volume ratio (Meirovitch et al., 1980).

Pestana et al. (2021) demonstrated that the more hydrophilic MC-LR had lower adsorption onto microparticles of PET, PE, PVC, and PS compared to the more hydrophobic MC-LF. Kamp et al. (2016) also showed greater adsorption of the hydrophobic analogues on plastic containers (adsorption MC-LF > -LA > -LR). In the current study, the most hydrophilic analogues have shown the lowest adsorption onto small sized PP. However, despite presenting the lowest adsorption, MC-RR displayed marked ($p < 0.0001$) adsorption onto small PP (83% ± 0, Fig. 2), compared to the control, after 48 h contact. MC-LA and -LY (not significantly different from each other; $p = 0.89$) also presented slightly less adsorption than the other analogues, nevertheless approximately 97% of MC-LA and -LY were adsorbed by small PP by the end of the experiment.

Hüffer and Hofmann (2016) have shown a strong relationship between the sorption coefficients of the microplastics and the hydrophobicity of the sorbates, suggesting the hydrophobic interactions were a major force driving the sorption of the microcystins on microplastics. Although the majority of the adsorption on microplastics are reported for hydrophobic contaminants (Velez et al., 2018; Yu et al., 2019), hydrophilic compounds have also been shown to interacted with microplastics, for example Ciprofloxacin, a hydrophilic antibiotic, was found to adsorb on PS and PVC microparticles (Liu et al., 2019).

3.5. Effect of the competition between the microcystin analogues

In cyanobacterial blooms, the presence of microcystin is often reported as a mixture of analogues. Most of the adsorption experiments reported in the scientific literature investigate individual compounds in contact with microplastics. Although it is important to understand how organic micropollutants interact individually with microplastics, this approach does not consider potential intramolecular adsorption competition. The current study demonstrated the presence intramolecular competition. MC-LR and -LF placed in contact with the smaller PP and PET particles individually, showed greater adsorption compared to the mixture (Fig. 4).

Small PP showed the greatest adsorption of the eight microcystin analogues either as a mixture or as individual. As an individual compound, $97 \pm 1\%$ of MC-LF was adsorbed after 2 h of contact with small PP, and undetectable in the solution after 4 h contact, which was the same as when present in a mixture. For MC-LR, within 2 h of contact with small PP, $92 \pm 3\%$ was adsorbed, with 99% of the initial concentration of MC-LR adsorbed by the end of the experiment (48 h, Fig. 4). In a mixture, MC-LR was undetectable after 4 h contact with small PP (Fig. 4). The rapid adsorption of both MC-LR and -LF onto the small PP prevented the evaluation of the competition behaviour of the microcystins, since MC-LR and -LF were rapidly and fully adsorbed both as a mixture and individually. On the other hand, for small PET, MC-LR in a mixture did not show adsorption, however, $5 \pm 1\%$ adsorption ($p < 0.04$) of MC-LR alone was observed. Similarly, a greater amount of MC-LF was adsorbed ($19 \pm 1\%$) as an individual compound when compared to MC-LF in a mixture ($11 \pm 2\%$) on small PET (Fig. 4).

The initial microcystin concentration may have affected the adsorption of microcystin on microplastics. Considering all analogues, the total microcystin concentration was 40 μg mL^{-1}, whereas, individually, the concentration of either MC-LR or -LF was 5 μg mL^{-1}. The concentration of the adsorbate has a similar type of effect as the pressure (Gabelman, 2017). Surface adsorption plays a more dominant role at lower adsorbate concentrations, on the other hand at higher adsorbate concentrations, partition (pore-filling) is more dominant (Velez et al., 2018), which can increase the amount adsorbed onto the microplastics. With a mixture of microcystins, small PET adsorbed 181.21 μg g^{-1} of the total microcystins (Fig. 3), while individually, MC-LR and -LF adsorbed approximately 24 μg g^{-1} and 60 μg g^{-1}, respectively. However, considering each analogue, lower amounts of MC-LR (3 μg g^{-1}) and -LF (52 μg g^{-1}) adsorbed onto small PET when in a mixture of microcystin. The decreased adsorption of MC-LF in a mixture indicated a potential competition between the most hydrophobic microcystin on the tested microplastics. In the microcystins mixture, MC-LW showed the greatest adsorption of the eight microcystin analogues onto small PET ($18 \pm 2\%$ after 48 h), followed by MC-LF ($11 \pm 2\%$). Similarly, MC-LW adsorbed 5 $\pm 1\%$ after 48 h, while MC-LF adsorbed $2 \pm 1\%$ onto large PET. The results suggested that out of all microcystin analogues tested, MC-LW and -LF were the most likely analogues to adsorb on small PET. Microcystin adsorption competition for binding sites on solid materials has been reported previously, with Wu et al. (2011) demonstrating a reduction of the amount of microcystin adsorbed onto sediments when in a solution with an increased concentration of dissolved organic matter. Medium characteristics such as pH, salinity, ionic strength, and dissolved organic matter have all been reported to affect the adsorption of organic compounds onto microplastics (Guo et al., 2019a; Wagstaff et al., 2022). Microplastic particle surfaces are negatively charged, therefore ions in the medium can bind electrostatically to the binding sites, disturbing the charge equilibrium of the surface (Atugoda et al., 2021).

3.6. Potential environmental impact

The co-occurrence of micropollutants (e.g. microcystins) and microplastics in aquatic systems is of environmental concern since
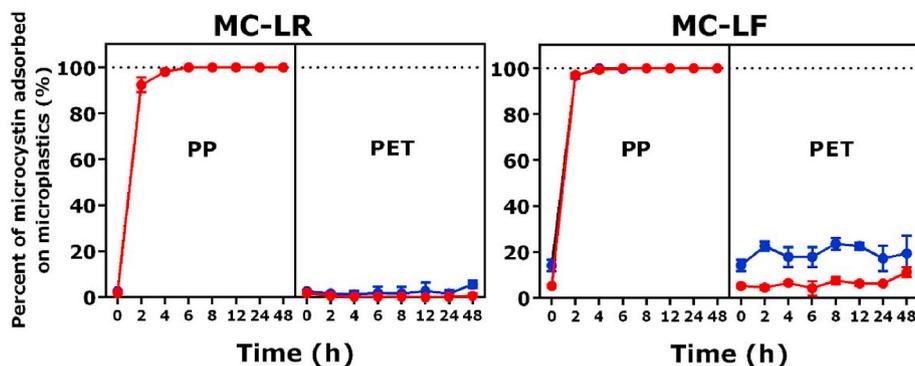
Fig. 4. Adsorption of MC-LR and -LF either as a mixture (●) or individually (●) on polypropylene (PP) and on polyethylene terephthalate (PET), both small (D$_{50}$ 8–28 μm) particle size. n = 3, errors bars = 1 SD. The individual (●) is not apparent in MC-LR and partly hidden in MC-LF due to the mixture plot (●).

contaminated plastic particles could act as a vector of micropollutants into the food web. In the current study, we elucidated the factors that govern the adsorption of microcystins onto microplastics. Three key factors determine the interaction of microcystin adsorption: hydrophobicity of the microcystin analogues, the size of the microplastic particles, and finally the polymer type.

In the past, hydrophobic compounds have shown increased adsorption onto microparticles (Velez et al., 2018). In the current study, the hydrophobic analogues MC-LW and -LF adsorbed in greater amounts onto all microplastic investigated. This is particularly concerning as a positive correlation between hydrophobicity and microcystin toxicity has been reported (Feurstein et al., 2011; Fischer et al., 2010). In an *in vivo* toxicity study conducted with the aquatic protozoan *Tetrahymena pyriformis*, MC-LF was 1.4–3.5 times more toxic than MC-LR (Ward and Codd, 1999). Further compounding this problem is the fact that MC-LR is frequently used in studies as a representative of the entire group of microcystins, despite the fact that it is not the most toxic microcystin analogue. As we have clearly demonstrated in the present study, other more hydrophobic microcystin analogues are more likely to adsorb onto microplastic particles compared to MC-LR. This finding is worrying as microplastic particles can potentially find their way into the food web and may lead to microcystins to become bioavailable.

Another factor affecting the adsorption onto microplastics is the size of the microparticles. In the current study we have demonstrated that the smaller the particle, the greater the adsorption potential. This observation is concerning as monitoring small particles in the aquatic system is challenging. The frequently used manta trawls for collection of microplastic particles do not retain particles smaller than 333 μm (Eriksen et al., 2013; Koelmans et al., 2019), therefore they are impractical for monitoring microplastic particles of the size used in the current study. Similarly, PP particles sized from 5 to 250 μm were the main microplastic type identified in supermarket-bought mussels in a study carried out in the UK (Li et al., 2018b). Additionally, smaller microplastics are more likely to be ingested by aquatic wildlife as demonstrated by Nematdoost Haghi and Banaee (2017) and Xia et al. (2020). In their studies they reported adult carp ingesting PE (<5 mm) and PVC (100–200 μm) microparticles. Additionally, low density microplastic like PP (0.9 g cm^{-3}) floats in the water column and could easily be mistaken as a planktonic food source compared to other, more dense polymers such as PET (1.29–1.40 cm^{-3}, figure S3) that might sediment, making PP more likely to be consumed by aquatic wildlife.

The third factor affecting adsorption is the type of polymer. Plastic polymers present varied properties that can influence the adsorbance of aquatic contaminants. In Europe, PP is the most commonly produced type of plastic (19.4%, Plastics Europe, 2020), consequently, it is also the microplastic type most reported in freshwater systems (Koelmans et al., 2019). PET represents 7.9% of the annual plastic demand in the European Union (Plastics Europe, 2020), and is in the top six

microplastic reported in the environment (Koelmans et al., 2019). We have demonstrated that PP showed greater adsorption of the microcystin analogues investigated compared to PET. However, the adsorption onto PET is still environmentally concerning since the more hydrophobic microcystins have been demonstrated to adsorb to it.

The factors affecting the adsorption behaviour of microcystin onto microplastics are interwoven and complex. Hydrophobicity of the microcystin analogue, particle size, and the polymer material all play roles to varying degrees. Based on our findings, a worst-case scenario would be the co-occurrence of hydrophobic microcystin analogues (MC-LW and -LF), and small particulate PP.

4. Conclusion

The findings presented in the current study have improved the understanding of the interaction of microplastics with natural pollutants in aqueous ecosystems. The hydrophobicity of microcystin analogue, polymeric material, and the size of the particles were key factors that influenced the adsorption of microcystins onto microplastics. Our findings suggest that highly toxic hydrophobic microcystin analogues have greater potential to be transported into the food chain by microplastics. The adsorption of hydrophobic microcystin is enhanced when in contact with small particles of rubbery polymers like PP. Furthermore, particle properties such as surface area and surface morphology can increase the adsorption potential of microplastics. Due to its high porosity and roughness, small PP microparticles can also act as a vector for hydrophilic analogues. For future research, it will be important to investigate how the water matrix influence the adsorption of microcystin, as well as the desorption behaviour of microcystins from different types and sizes of microplastics under different conditions, especially those present in the gastrointestinal tract of aquatic animals.

Author statement

Diana S. Moura: Investigator, Writing – original draft, Visualization, Carlos J. Pestana: Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Project administration, Colin F. Moffat: Resources, Writing – review & editing, Supervision, Jianing Hui: Methodology, John T. S. Irvine: Methodology, Christine Edwards: Methodology, Linda A. Lawton: Conceptualization, Writing – review & editing, Supervision, Project administration

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.119135.

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1   Supporting Information for

2   **Adsorption of cyanotoxins on polypropylene and**
3   **polyethylene terephthalate: microplastics as vector**
4   **of eight microcystin analogues**

5

6   Diana S. Moura[a],*, Carlos J. Pestana[a], Colin F. Moffat[a], Jianing Hui[b], John T.

7   S. Irvine[b] Christine Edwards[a], Linda A. Lawton[a]

8   [a] School of Pharmacy and Life Sciences, Robert Gordon University,
9   Aberdeen, AB10 7GJ, UK

10   [b] School of Chemistry, University of St Andrews, North Haugh, St Andrews,
11   Scotland, KY16 9ST, UK.

12   * Corresponding Author. Tel. +44 1224 2837. E-mail address: d.souza-
13   moura1@rgu.ac.uk

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S1 Material and Methods

S1.1 Chemical structure of the microcystin and its amino acids

		Polarity	Hydropathy index*
Alanine (A)		Nonpolar	1.8
Arginine (R)		Polar	-4.5
Leucine (L)		Nonpolar	3.8
Phenylalanine (F)		Nonpolar	2.8
Tryptophan (W)		Nonpolar	-0.9
Tyrosine (Y)		Polar	-1.3
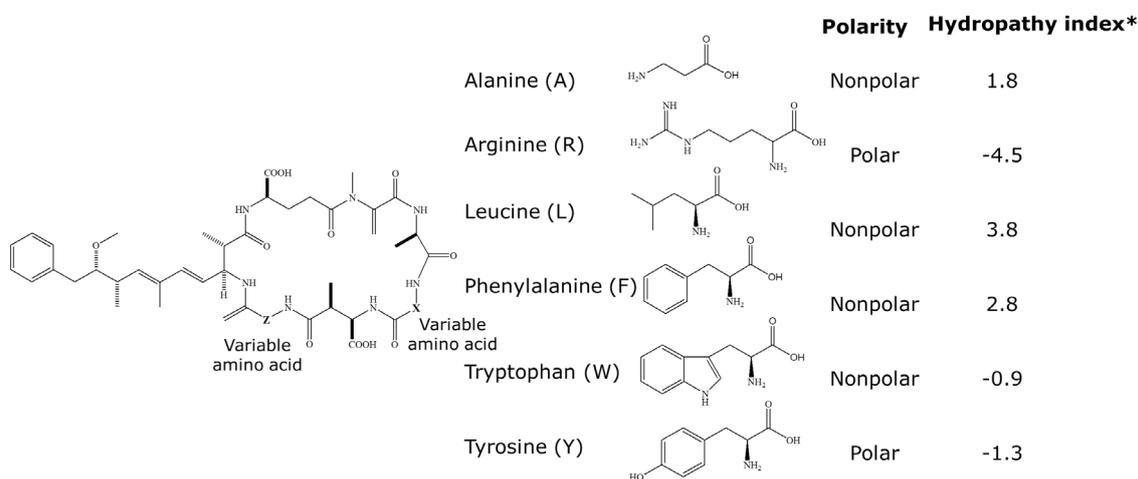
Figure S1: General chemical structure of microcystin where the letters X and Z represent the locations of the variable amino acids, and chemical structure of amino acids that comprise the microcystin analogues used in the current study. *Hydropathy Index is a number representing the hydrophobic or hydrophilic properties of the side chain of an amino acid (Kyte and Doolittle, 1982).

S1.2 Determination of microcystin analogue concentration

Table S1: Solvent gradient used in the HPLC analysis of microcystin analogues. Solvent A: ultra-pure water (18.2 MΩ) + 0.05% (v/v) TFA; Solvent B: acetonitrile + 0.05% (v/v) TFA. The Gradient elution profile is represented by figures, 1 means a step gradient and 6 means a linear gradient.

Time (min)	% Solvent A	% Solvent B	Gradient elution profile
0	70	30	-
11	40	60	6
12	0	100	1
13	70	30	1
15	70	30	1

41

43 **Figure S2:** HPLC chromatogram of a mixture containing eight microcystin
44 analogues (MC-RR, -YR, -LR, -WR, -LA, -LY, -LW, and -LF) using the method used
45 in this study (see section 2.3, table S1). Wavelength set at 238 nm.
46
47 **S1.3 Characterisation of microplastics**

Polypropylene (PP)
Density: 0.90 g cm^{-3}
Tg: 0 °C

Polyethylene terephthalate (PET)
Density: 1.29-1.40 g cm^{-3}
Tg: 69 °C

49 **Figure S3:** Monomer unit and selected physicochemical properties of polypropylene
50 and polyethylene terephthalate (Alimi et al., 2018; Anderson et al., 2016; Misumi,
51 2011).

52

53

55 **Figure S4:** FT-IR spectra of the microplastics polypropylene (PP), and polyethylene
56 terephthalate (PET).
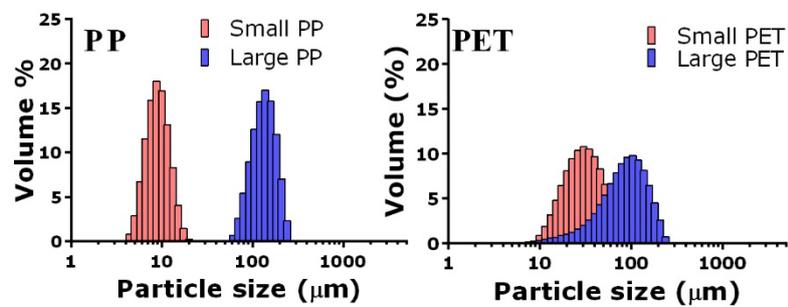
58 **Figure S5:** Particle size analysis of polypropylene (PP) and, and polyethylene
59 terephthalate (PET) two size ranges. The red colour represents the smaller size
60 range acquired, while the blue colour represents the larger size range.

61
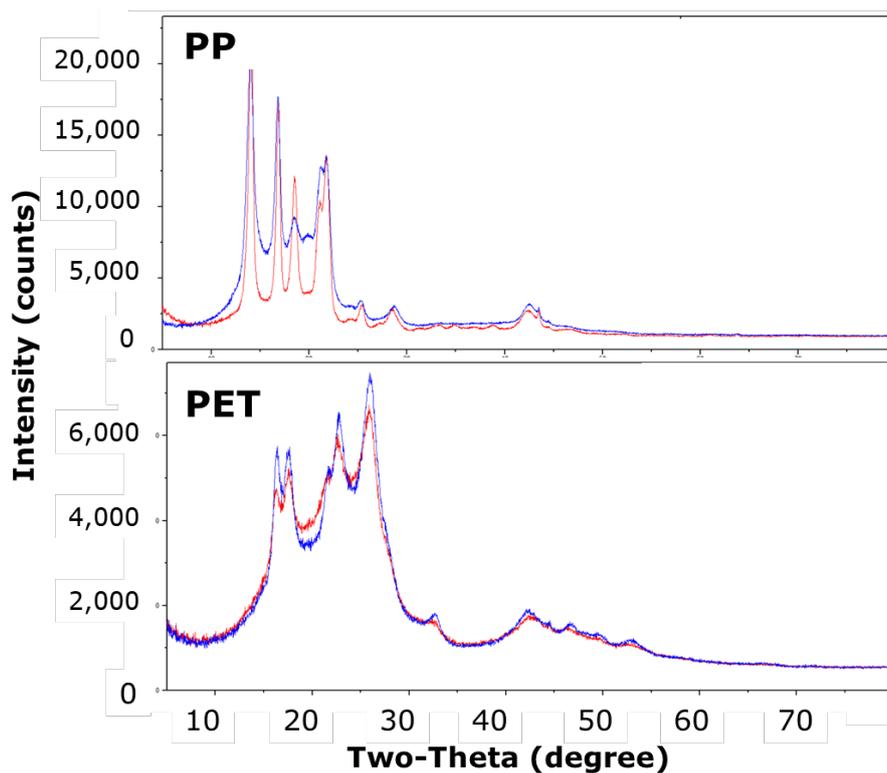
63 **Figure S6:** X-Ray diffraction (XRD) patterns of polypropylene (PP), and

64 polyethylene terephthalate (PET) both sizes. The red line represents the small

65 plastic particles, while the blue line represents the large plastic particles.

66 **S1.4 Statistical tests**

67 After applying a Shapiro-Wilk normality test to the experimental data,

68 normal distribution of the data could not be assumed, thus a Wilcoxon rank

69 sum test was performed to evaluate whether there is a significant difference

70 between each microcystin congeners with their respective control (**Table**

71 **S2**). Further, this statistic test was also applied to verify the difference of

72 each microcystin variant with each microplastic type and size, e.g.

73 polyethylene terephthalate (PET) and polypropylene (PP) at the small

74 particles size, and both at the large particles (**Table S2** to **Table S5**).

75 Wilcoxon rank sum test was also applied to the data from the second

76 experiment where MC-LF alone was tested in a comparison study between

77 virgin large PET microplastic particles and PET processed in the laboratory

to evaluate whether there is a difference on the adsorption capacity of the

plastic (**Table S6**).

Table S2: Wilcoxon rank sum test comparing the adsorption with each microplastic for each size with each microcystin analogue control. The significance level is set on 0.05. The colour red represents $p > 0.05$, therefore, not significant different, the colour green represents $p < 0.05$, therefore, significant different. Polyethylene terephthalate (PET), polypropylene (PP).

Solution with plastic	Control							
	MC-RR	MC-YR	MC-LR	MC-WR	MC-LA	MC-LY	MC-LW	MC-LF
PET 15-25 µm	0.69000	0.36000	0.49000	0.00043	0.18000	0.00090	0.00000	0.00000
PET 100 µm	0.89000	0.56000	0.46000	0.02500	0.09800	0.55000	0.00000	0.00000
PP 15-25 µm	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
PP 100 µm	1.00000	0.42000	0.38000	0.14000	0.21000	0.75000	0.00009	0.00000

Table S3: Wilcoxon rank sum test comparing the adsorption data of eight microcystin congeners with each other for adsorption onto polypropylene (PP) at the size 15-25 µm. The significance level is set on 0.05. The colour red represents $p > 0.05$, therefore, not significant different, the colour green represents $p < 0.05$, therefore, significant different.

PP 15-25 µm	MC-RR	MC-YR	MC-LR	MC-WR	MC-LA	MC-LY	MC-LW	MC-LF
MC-RR	-	-	-	-	-	-	-	-
MC-YR	0.00000	-	-	-	-	-	-	-
MC-LR	0.00000	0.67000	-	-	-	-	-	-
MC-WR	0.00000	0.00000	0.00000	-	-	-	-	-
MC-LA	0.00000	0.00000	0.00000	0.00001	-	-	-	-
MC-LY	0.00000	0.00000	0.00000	0.00000	0.89000	-	-	-
MC-LW	0.00000	0.00001	0.00001	0.00000	0.35000	0.35000	-	-
MC-LF	0.00000	0.00000	0.00000	0.00000	0.89000	0.92000	0.37000	-

Table S4: Wilcoxon rank sum test comparing the adsorption of the eight microcystin variants with each other for polypropylene (PP) at the size 100 µm. The significance level is set on 0.05. The significance level is set on 0.05. The colour red represents $p > 0.05$, therefore, not significant different, the colour green represents $p < 0.05$, therefore, significant different.

PP 100 µm	MC-RR	MC-YR	MC-LR	MC-WR	MC-LA	MC-LY	MC-LW	MC-LF
MC-RR	-	-	-	-	-	-	-	-
MC-YR	0.68984	-	-	-	-	-	-	-
MC-LR	0.68984	0.89362	-	-	-	-	-	-
MC-WR	0.21218	0.02923	0.02160	-	-	-	-	-
MC-LA	0.53300	0.68900	0.70000	0.01300	-	-	-	-
MC-LY	0.76299	1.00000	0.89362	0.02160	0.53300	-	-	-
MC-LW	0.06717	0.00042	0.00007	0.83579	0.00004	0.00012	-	-

MC-LF	0.02160	0.00003	0.00002	0.60483	0.00001	0.00002	0.53300	-

Table S5: Wilcoxon rank sum test comparing the adsorption of the eight microcystin variants with each other for polypropylene (PET) at the size 15-25 µm. The significance level is set on 0.05. The colour red represents $p > 0.05$, therefore, not significant different, the colour green represents $p < 0.05$, therefore, significant different.

PET 15-25 µm	MC-RR	MC-YR	MC-LR	MC-WR	MC-LA	MC-LY	MC-LW	MC-LF
MC-RR	-	-	-	-	-	-	-	-
MC-YR	0.61754	-	-	-	-	-	-	-
MC-LR	0.68984	0.87338	-	-		-	-	
MC-WR	0.00024	0.00004	0.00008	-	-	-	-	-
MC-LA	0.76335	0.22554	0.20572	0.00000	**-**	**-**	**-**	**-**
MC-LY	0.76335	0.76299	1.00000	0.00000	0.17778	-	-	-
MC-LW	0.00000	0.00000	0.00000	0.25820	0.00000	0.00000	-	-
MC-LF	0.00006	0.00000	0.00001	0.61754	0.00000	0.00000	0.00018	-

Table S6: Wilcoxon rank sum test comparing the adsorption of the eight microcystin variants with each other for polypropylene (PET) at the size 100 µm. The significance level is set on 0.05. The colour red represents $p > 0.05$, therefore, not significant different, the colour green represents $p < 0.05$, therefore, significant different.

PET 100 µm	MC-RR	MC-YR	MC-LR	MC-WR	MC-LA	MC-LY	MC-LW	MC-LF
MC-RR	-	-	-	-	-	-	-	-
MC-YR	0.61754	-	-	-	-	-	-	-
MC-LR	0.68984	0.87338	-	-		-	-	
MC-WR	0.00024	0.00004	0.00008	-	-	-	-	-
MC-LA	0.76335	0.22554	0.20572	0.00000	**-**	**-**	**-**	**-**
MC-LY	0.76335	0.76299	1.00000	0.00000	0.17778	-	-	-
MC-LW	0.00000	0.00000	0.00000	0.25820	0.00000	0.00000	-	-
MC-LF	0.00006	0.00000	0.00001	0.61754	0.00000	0.00000	0.00018	-