1 **Supporting Information:**

2 **Potentially poisonous plastic particles: microplastics as vector for**

3 **cyanobacterial toxins microcystin-LR and –LF**

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15 The supporting information contains 5 figures, 5 tables, and 2 equations on 14

16 pages.

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S1 Plastics selected for this study

Four plastics that represent typical plastic pollutants were selected for this

study: polystyrene (PS), polyvinyl chloride (PVC), polyethylene (PE), and

polyethylene terephthalate (PET) (Figure S1). These polymers were selected for

their widespread application in food packaging and personal care products.

Further, several studies have identified these polymers as the most frequently

encountered in both marine and fresh water aquatic systems[1–4].

Polystyrene (PS)   Polyvinyl chloride (PVC)   Polyethylene (PE)   Polyethylene terephthalate (PET)
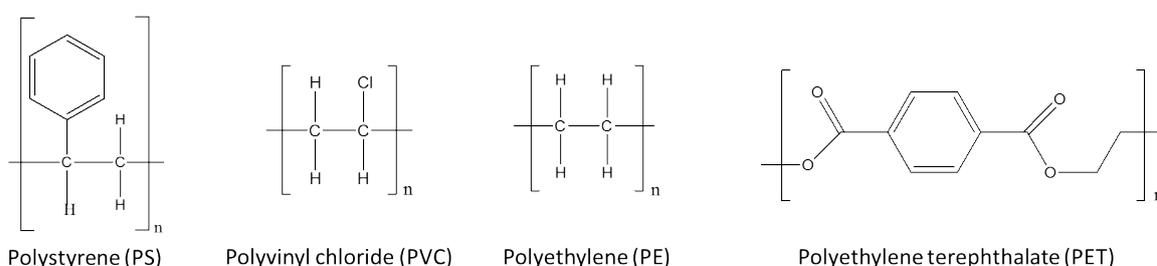
Figure S1: Chemical structures of plastic monomers selected as sorbents in this study.
Representing four of the most commonly encountered plastic pollutants in the
environment.

Additionally, the selected plastics present with a chemically diverse set of

monomers including aromatic rings (PS and PET) and halogen moieties (PVC)

which can affect adsorption behaviour and sorbent-sorbate interaction[5].

S2: Suppliers of chemicals and instruments

S2.1 Chemicals and materials

All organic solvents (methanol and acetonitrile) were purchased from Fisher

Scientific (UK) and were of analytical grade. Trifluoroacetic acid (TFA) used as

ion pairing agent in high performance liquid chromatography (HPLC) was

77 purchased from Fisher Scientific (UK). Microcystin-LR and -LF reference

78 materials were isolated from laboratory cultures with >90% purity. Ultrapure

79 water was produced with an Elga Water Purification System to a resistivity of

80 18.2 MΩ. Chemical solutions for the adjustment of the pH (nitric acid and

81 sodium hydroxide) were purchased from Sigma Aldrich (UK). The chemicals for

82 the preparation of artificial freshwater ($CaCl_2.2H_2O$, $MgSO_4.2H_2O$, $NaHCO_3$, and

83 KCl) were purchased from Fischer Scientific (UK). Polystyrene, polyethylene, and

84 polyethylene terephthalate were received as pellets (3-5 mm diameter), while

85 polyvinyl chloride was received as 1 m rods (5 mm diameter). All plastics were

86 purchased from Goodfellow (UK). Fourier Transformer Infrared

87 Spectrophotometry (FT-IR) were used characterised the plastics received to

88 establish the polymer material received (Figure S2). The spectra confirmed the

89 polymer type of the plastics used in the current study.
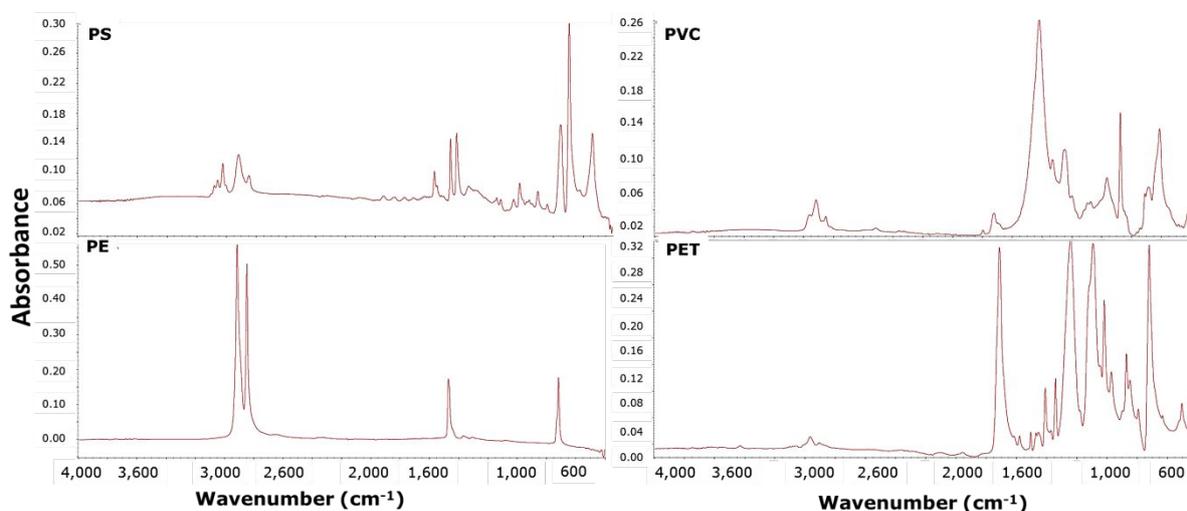
90
91 **Figure S2:** FT-IR spectrum of polystyrene (PS), polyvinyl chloride (PVC), polyethylene
92 (PE), and polyethylene terephthalate (PET).
93

S2.2 Devices and auxiliaries

95 A Nicolet iS10 FT-IR Spectrometer from Thermo Fisher Scientific with OMNIC

96 Spectra Software was used to analyse the material received from GoodFellow,

97 UK. The FT-IR scanning wavenumber was set from 400 to 4000 cm^{-1}. The

98   sample was scanned 32 times, the resolution was set at 8 cm^{-1}, no correction

99   was applied.

100   HPLC analysis was performed by using a Waters 2695 Separation Module. High

101   resolution photodiode array detection was performed with a Waters 2996

102   Photodiode Array Detector (PDA). Separation of analytes was performed with a

103   Symmetry C18 column 2.1 mm (inner diameter) x 150 mm with a 5 μm particle

104   size (all Waters, UK). The mobile phases used were ultrapure water and

105   acetonitrile, both 0.05% TFA. Chromatography was achieved with a linear

106   gradient from 15 to 75% acetonitrile over 10 minutes, followed by a solvent

107   wash and equilibration. Column temperature was set to 40˚C and the flowrate

108   applied was 0.3 mL min^{-1}. The resolution of the PDA was set to 1.2 nm and data

109   was acquired over a range of 200 to 400 nm. The limit of quantification achieved

110   by this method was 0.1 μg mL^{-1}. The measurement of pH was performed with a

111   Five Easy pH probe (Mettler Toledo, USA). Experiments were conducted in an

112   incubation chamber with an orbital shaker (Thermofisher MaxQ 6000, UK) at

113   25˚C with horizontal agitation at 250 rpm. Size reduction of the plastics was

114   achieved in an industrial stainless-steel blender (Waring, USA).

115   Scanning electron microscopy of the 0.09-0.125 mm plastic samples was

116   performed on an EVO LS10 (Carl Zeiss Ltd, Germany) scanning electron

117   microscope. Samples were mounted on aluminium stubs with double sides

118   carbon adhesive pads and then sputter coated in a gold and palladium mixture.

119   Scanning electron microscopy was performed under stable pressure, with an

120   acceleration voltage of 25 kV and a working distance of 6.5 to 8 mm.

121   3D-surface MALDI imaging was performed using an autofocusing AP-SMALDI5 AF

122   high-resolution MALDI imaging ion source (TransMIT GmbH, Germany),

123   operating at atmospheric pressure and coupled to a Q Exactive HF Orbitrap mass

spectrometer (Thermo Fisher Scientific, Germany). The sample was irradiated with 50 UV-laser pulses ($\lambda = 343$ nm) per pixel at a frequency of 100 Hz. The dedicated autofocusing system enabled keeping the laser focus diameter, fluence and ablation spot size constant across the non-flat sample surface by adjusting the sample stage position according to the sample's height profile for each measurement spot. Samples were scanned with 12 µm step size and the target voltage was set to 3 kV. The mass spectrometer was operated in positive-ion mode in a mass-to-charge-number (m/z) range of 350 to 1200 at a mass resolution of 240,000 at m/z 200. Internal lock-mass calibration was performed by using a signal of a DHB matrix cluster ($[5DHB-4H_2O+NH_4]^+$, m/z 716.12461), resulting in a mass accuracy of better than 2 ppm for the entire image. The ion injection time was set to 500 ms. The S-lens level was set to 100 arbitrary units, and the capillary temperature was 250 °C.

A Malvern Zetasizer Nano-ZS ZEN3600 was used to measure the electrostatic charge of PET, PVC, PE, and PS in the experimental medium under five different pHs. A solution containing 20 g L^{-1} of each plastic in AFW adjusted to pH 3, pH 5, pH 7, pH 9, and pH 10 was prepared. The pH 2 and pH 11 were not evaluated due the zeta potential cell compatibility (Malvern Zetasizer cell, DTS1070). Before each analysis, the cell was washed with filtered (0.22 µm) methanol followed by filtered (0.22 µm) ultra-pure water (18.2 MΩ). Later, the solution with microplastics was placed in the zeta cell carefully evaluating the existence of bubbles. Three measurements were performed of each sample.

S3: 3D-surface MALDI imaging of MC-LF bound to polystyrene (PS) microplastic particles

149   The optical image of the matrix-covered microplastic surface after analysis is

150   depicted in Figure S3b, demonstrating uniform laser ablation spots throughout

151   the sample height profile (see Figure S3c). PS microplastic samples showed

152   height variations up to 155 µm (see Figure S4). To overcome the limitation of

153   non-flat surfaces in microplastic samples, a MALDI imaging ion source with the

154   capabilities to maintain uniform laser foci on rough surfaces is essential ("3D-

155   surface mode").

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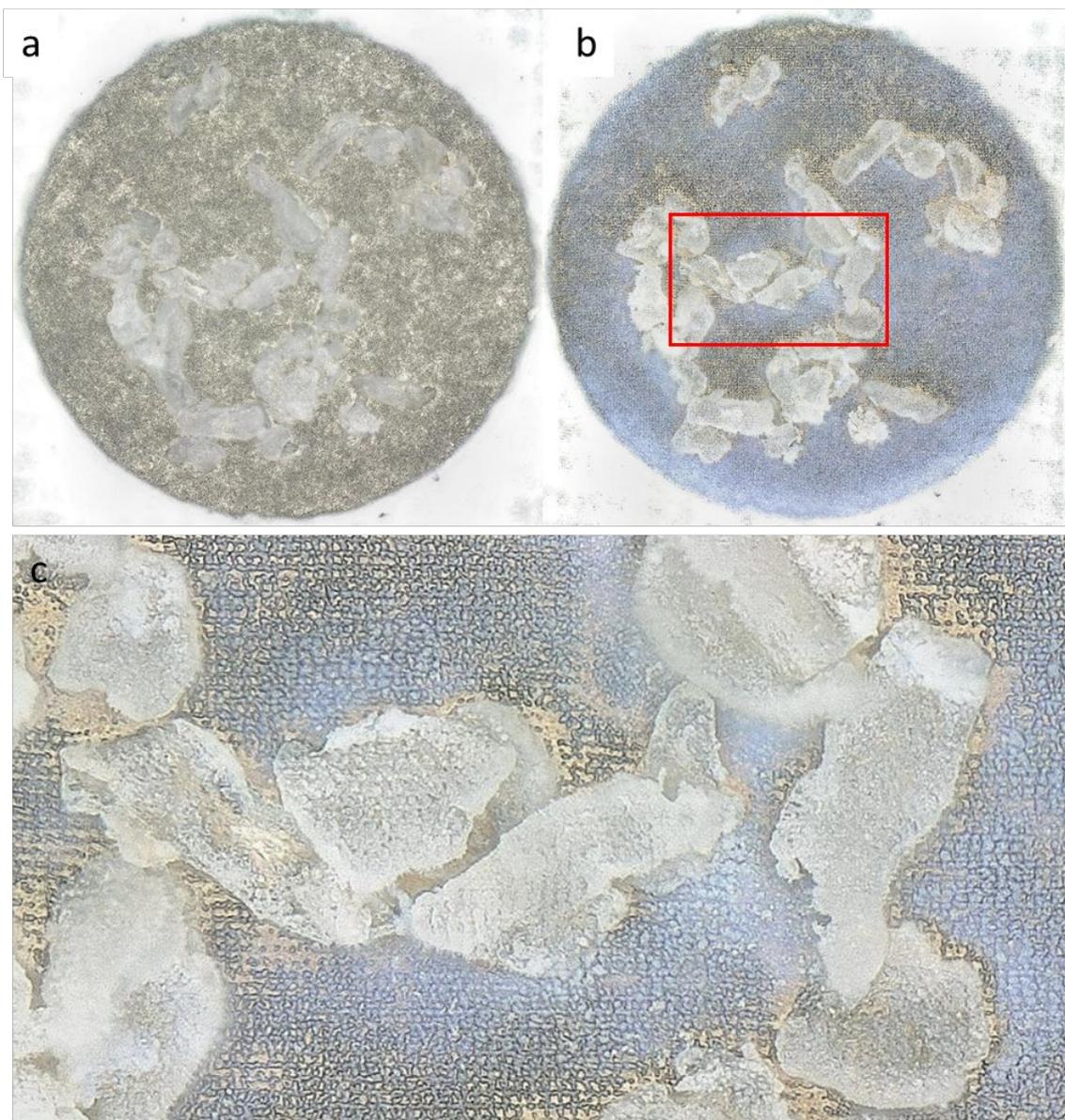
157

158 **Figure S3:** (a) Optical image of a PS microplastic sample after matrix application. (b)
159 Optical image of the same PS microplastic sample after MALDI MSI analysis. (c)
160 Magnification of the marked area, showing uniform laser ablation marks throughout the
161 sample height profile.
162
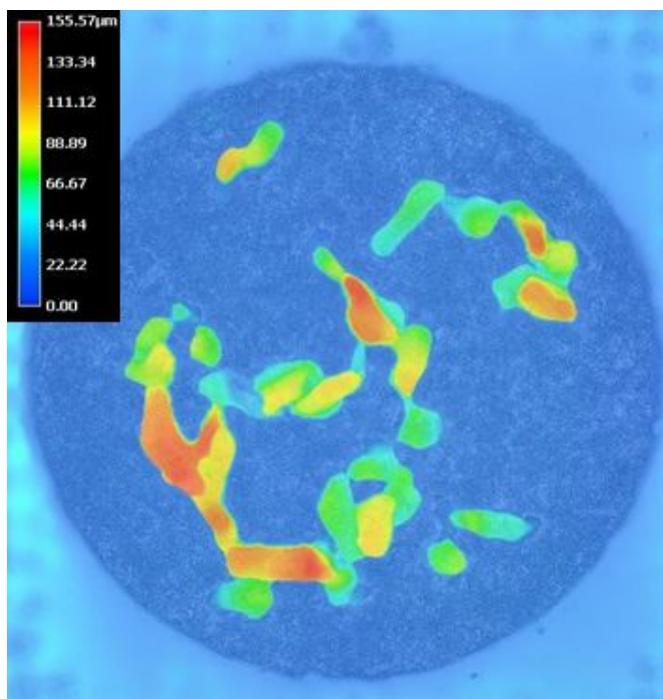
163
164 **Figure S4:** Topography image of PS microplastic showing height variations up to 155 µm.
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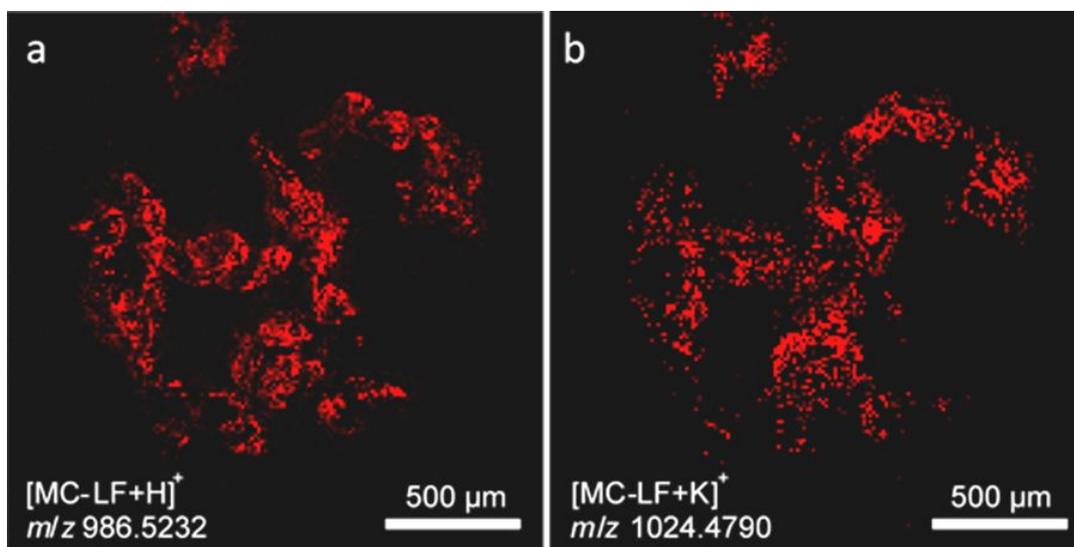
167 **Figure S5:** 3D-surface MALDI MS images showing the spatial distributions of (a) [MC-
168 LF+H]+ at *m/z* 986.5232 and (b) [MC-LF+K]+ at *m/z* 1024.4790 in red. MS images were
169 generated with 170x174 pixel, 12 µm * 12 µm pixel size, image bin width: Δ(*m/z*) = 0.01.
170 The scale bars are 500 µm.

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S4: Prediction of toxin concentration per particle of plastic

Having established the concentration of MC-LR and –LF after 48 h of exposure

(table S1) allows for the determination of the amount of plastic per particle,

allowing for a number of assumptions.

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Table S1: Concentration of MC-LR and –LF per gram of plastic after 48 hours of
exposure. (Experimental conditions: 10 g L^{-1} plastic, 5 µg L^{-1} MC, horizontal agitation in
the dark, pH7, 25 °C).

| Plastic	MC-LR (µg g^{-1})	MC-LF (µg g^{-1})
PET	23.61	142.31
PVC	0	0
PE	13.85	96.69
PS	1.06	91.23

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Determination was performed for pH7 results only, as this is the most

environmentally relevant pH condition tested and the smallest particle size

employed in the study. As the particle preparation of blending with subsequent

sieving yields particle size ranges rather than single sizes, the first assumption

was an average particle size of 0.1075 mm from the 0.09 to 0.125 mm range. In

order to be able to calculate the amount of toxin per particle, the volume of the

particle needs to be determined (Equation S1), this can only be done by

assuming perfect sphericity of the particles.

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Equation S1: $$V = \frac{4}{3}\pi r^2$$

where:

V = volume of the particle; r = radius of the plastic particle

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198 Then using the densities of the different types of plastic (as stated by the

199 supplier) the weight of each particle can be determined (Equation S2).

200

201 Equation S2: $$\rho = \frac{m}{V}$$

202 where:

203 ρ = density of the plastic; m =mass of the particle; V = volume of the particle

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205 Combining the information of the amount of MCs per mass for each of the four

206 plastics tested, the amount of toxin per plastic particle can be determined (Table

207 S2).

208

209 **Table S2:** Amount of MC-LR and –LF per plastic particle after 48 hours of exposure.
210 (Experimental conditions: 10 g L^{-1} plastic, 5 µg L^{-1} MC, horizontal agitation in the dark,
211 pH 7, 25 $^{\circ}$C).
212

Plastic	MC-LR (pg particle^{-1})	MC-LF (pg particle^{-1})
PET	0	0
PVC	12.4	86.7
PE	0.64	55.1
PS	23.6	127

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214

215 S5: Evaluation of the electrostatic charge of each type of microplastics

216 under five pH conditions

217 **Table S3:** Zeta potential measurement (mV) of polyethylene terephthalate (PET),
218 polyvinyl chloride (PVC), polyethylene (PE), and polystyrene (PS) in artificial freshwater
219 at pH 3, pH 5, pH 7, pH 9, and pH 10.
220

Plastic	pH 3	pH 5	pH 7	pH 9	pH 10
			mV		
PET	0.08	-0.06	-0.42	-2.50	-5.66
PVC	0.06	-6.20	-10.96	-10.89	-2.96
PE	-10.88	-5.62	-12.63	-0.19	0.17
PS	-4.17	-2.90	-24.37	-11.88	0.03

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S6: Statistical analysis

S6.1: Significance testing performed using T-test comparing samples

with the control (no plastics)

The sample data (n=3) were compared with the control data (n=3) to evaluate whether the microcystin adsorption occurred was significant. *P*-values lower than 0.05 (5 percent) were considered as significant different from the control, therefore the occurrence of adsorption was assumed (Table S4).

Table S4: *P*-values of t-test significance testing comparing the samples (n=3) with the control (n=3, no plastics). *P*-values > 0.05 (red) adsorption cannot be assumed, *p*-values < 0.05 (green) adsorption can be assumed. SZ-A represents the small size (0.09-0.125 mm), SZ-B represents the medium size (0.25-0.50 mm), SZ-C represents the large size (1-5 mm), and the letter C represents the control.

PET	pH 2		pH 5		pH 7		pH 9		pH 11	
	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF
SZ-A(2h)xC(2h)	0.11	0.01	0.61	0.83	0.93	0.32	0.13	0.72	0.23	0.22
SZ-B(2h)xC(2h)	0.20	0.23	0.74	0.87	0.08	0.39	0.10	0.11	0.28	0.61
SZ-C(2h)xC(2h)	0.60	0.29	0.73	0.64	0.11	0.20	0.11	0.40	0.19	0.16
SZ-A(6h)xC(6h)	0.93	0.02	0.30	0.89	0.54	0.50	0.08	0.09	0.36	0.22
SZ-B(6h)xC(6h)	0.95	0.55	0.30	0.24	0.29	0.79	0.08	0.92	0.30	0.85
SZ-C(6h)xC(6h)	0.56	0.48	0.27	0.23	0.25	0.14	0.09	0.49	0.39	0.65
SZ-A(12h)xC(12h)	0.01	0.00	0.11	0.14	0.01	0.09	0.01	0.02	0.04	0.03
SZ-B(12h)xC(12h)	0.63	0.14	0.67	0.13	0.13	0.89	0.23	0.80	0.89	0.08
SZ-C(12h)xC(12h)	0.55	0.45	0.57	0.04	0.00	0.37	0.34	0.79	0.11	0.13
SZ-A(24h)xC(24h)	0.31	0.01	0.41	0.62	0.62	0.48	0.36	0.50	0.22	0.64
SZ-B(24h)xC(24h)	0.34	0.75	0.65	0.04	0.52	0.75	0.10	0.44	0.27	0.54
SZ-C(24h)xC(12h)	0.10	0.02	0.26	0.01	0.36	0.34	0.20	0.59	0.31	0.18
SZ-A(48h)xC(48h)	0.25	0.06	0.07	0.35	0.56	0.96	0.13	0.18	0.47	0.38
SZ-B(48h)xC(48h)	0.39	0.01	0.23	0.09	0.38	0.05	0.24	0.11	0.22	0.69
SZ-C(48h)xC(48h)	0.99	0.22	0.17	0.63	0.26	0.06	0.15	0.03	0.16	0.60
PE	pH 2		pH 5		pH 7		pH 9		pH 11	
	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF
SZ-A(2h)xC(2h)	0.02	0.00	0.15	0.07	0.21	0.16	0.91	0.03	0.10	0.03
SZ-B(2h)xC(2h)	0.10	0.01	0.03	0.70	0.46	0.85	0.35	0.15	0.25	0.22
SZ-C(2h)xC(2h)	0.23	0.17	0.08	0.80	0.08	0.56	0.13	0.98	0.40	0.25
SZ-A(6h)xC(6h)	0.25	0.05	0.69	0.04	0.34	0.05	0.25	0.00	0.81	0.00

	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF
SZ-B(6h)xC(6h)	0.36	0.03	0.23	0.02	0.49	0.24	0.38	0.04	0.28	0.02
SZ-C(6h)xC(6h)	0.47	0.79	0.17	0.05	0.27	0.01	0.12	0.07	0.45	0.12
SZ-A(12h)xC(12h)	0.01	0.00	0.17	0.03	0.02	0.00	0.02	0.00	0.01	0.00
SZ-B(12h)xC(12h)	0.36	0.07	0.94	0.01	0.37	0.25	0.48	0.07	0.06	0.32
SZ-C(12h)xC(12h)	0.78	0.64	0.72	0.02	0.12	0.70	0.28	0.87	0.10	0.14
SZ-A(24h)xC(24h)	0.12	0.00	0.72	0.01	0.17	0.01	0.35	0.00	0.75	0.00
SZ-B(24h)xC(24h)	0.18	0.02	0.84	0.24	0.82	0.01	0.16	0.03	0.53	0.02
SZ-C(24h)xC(12h)	0.25	0.33	0.75	0.21	0.35	0.86	0.02	0.60	0.63	0.24
SZ-A(48h)xC(48h)	0.07	0.03	0.28	0.05	0.83	0.00	0.51	0.01	0.31	0.02
SZ-B(48h)xC(48h)	0.03	0.09	0.71	0.09	0.70	0.00	0.24	0.18	0.16	0.03
SZ-C(48h)xC(48h)	0.05	0.19	0.09	0.12	0.07	0.06	0.20	0.05	0.04	0.12

PVC	pH 2		pH 5		pH 7		pH 9		pH 11	
	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF
SZ-A(2h)xC(2h)	0.00	0.01	0.41	0.07	0.13	0.08	0.89	0.01	0.11	0.06
SZ-B(2h)xC(2h)	0.07	0.03	0.10	0.80	0.18	0.47	0.28	0.15	0.48	0.34
SZ-C(2h)xC(2h)	0.05	0.16	0.02	0.49	0.10	0.34	0.10	0.02	0.37	0.52
SZ-A(6h)xC(6h)	0.01	0.03	0.50	0.05	0.13	0.01	0.26	0.00	0.59	0.02
SZ-B(6h)xC(6h)	0.68	0.06	0.26	0.11	0.91	0.22	0.66	0.05	0.51	0.05
SZ-C(6h)xC(6h)	0.22	0.68	0.28	0.08	0.68	0.69	0.11	0.02	0.34	0.65
SZ-A(12h)xC(12h)	0.03	0.01	0.24	0.04	0.01	0.00	0.01	0.01	0.00	0.00
SZ-B(12h)xC(12h)	0.41	0.07	0.91	0.01	0.79	0.24	0.45	0.08	0.21	0.01
SZ-C(12h)xC(12h)	0.54	0.20	0.84	0.03	0.82	0.05	0.79	0.10	0.19	0.07
SZ-A(24h)xC(24h)	0.28	0.00	0.79	0.01	0.09	0.06	0.02	0.01	0.52	0.00
SZ-B(24h)xC(24h)	0.08	0.00	0.65	1.00	0.71	0.04	0.15	0.11	0.52	0.01
SZ-C(24h)xC(12h)	0.12	0.05	0.64	0.11	0.54	0.50	0.26	0.10	0.41	0.26
SZ-A(48h)xC(48h)	0.37	0.00	0.40	0.03	0.17	0.00	0.04	0.00	0.83	0.01
SZ-B(48h)xC(48h)	0.15	0.04	0.39	0.49	0.87	0.17	0.36	0.11	0.11	0.17
SZ-C(48h)xC(48h)	0.50	0.17	0.27	0.00	0.37	0.78	0.43	0.02	0.07	0.14

PS	pH 2		pH 5		pH 7		pH 9		pH 11	
	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF	MC-LR	MC-LF
SZ-A(2h)xC(2h)	0.02	0.00	0.08	0.02	0.00	0.02	0.07	0.01	0.51	0.00
SZ-B(2h)xC(2h)	0.91	0.03	0.66	0.19	0.52	0.71	0.21	0.05	0.17	0.02
SZ-C(2h)xC(2h)	0.96	0.37	0.29	0.90	0.10	0.48	0.12	0.30	0.14	0.32
SZ-A(6h)xC(6h)	0.23	0.06	0.30	0.01	0.05	0.01	0.02	0.00	0.18	0.00
SZ-B(6h)xC(6h)	0.45	0.02	0.90	0.89	0.66	0.06	0.93	0.00	0.40	0.10
SZ-C(6h)xC(6h)	0.47	0.12	0.50	0.02	0.23	0.54	0.15	0.09	0.59	0.11
SZ-A(12h)xC(12h)	0.05	0.00	0.47	0.02	0.01	0.00	0.01	0.00	0.01	0.00
SZ-B(12h)xC(12h)	0.47	0.04	0.77	0.02	0.50	0.13	0.76	0.02	0.21	0.10
SZ-C(12h)xC(12h)	0.53	0.41	0.37	0.08	0.16	0.84	0.28	0.08	0.19	0.04
SZ-A(24h)xC(24h)	0.05	0.00	0.04	0.00	0.02	0.00	0.03	0.00	0.17	0.00
SZ-B(24h)xC(24h)	0.21	0.00	0.44	0.55	0.84	0.35	0.25	0.03	0.28	0.07
SZ-C(24h)xC(12h)	0.38	0.64	0.32	0.52	0.42	0.11	0.29	0.45	0.35	0.78
SZ-A(48h)xC(48h)	0.09	0.00	0.05	0.01	0.01	0.00	0.01	0.00	0.98	0.02
SZ-B(48h)xC(48h)	0.19	0.02	0.50	0.26	0.50	0.02	0.96	0.08	0.08	0.09
SZ-C(48h)xC(48h)	0.22	0.27	0.88	0.08	0.37	0.38	0.82	0.34	0.34	0.41

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S6.2: Pearson correlation testing

Table S5: Pearson correlation coefficients (R^2) evaluating the correlation between the plastic type, MC-LR and -LF adsorption after 48 h contact, average sorption capacity ranking, and glass transition temperature

	PLASTIC	MC-LR adsorption	MC-LF adsorption	Average sorption capacity ranking	Glass transition temperature
PLASTIC	1.00				
MC-LR adsorption	0.96	1.00			
MC-LF adsorption	0.94	0.80	1.00		
Average sorption capacity ranking	-0.68	-0.47	-0.89	1.00	
Glass transition temperature	0.37	0.60	0.02	0.43	1.00

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