Microplastics profile in constructed wetlands: Distribution, retention and implications

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Submitted to:

Environmental Pollution

Date of re-submission:

2 August 2022

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Abstract

Wastewater and stormwater are both considered as critical pathways contributing microplastics (MPs) to the aquatic environment. However, there is little information in the literature about the potential influence of constructed wetlands (CWs), a commonly used wastewater and stormwater treatment system. This study was conducted to investigate the abundance and distribution of MPs in water and sediment at five CWs with different influent sources, namely stormwater and wastewater. The MP abundance in the water samples ranged between 0.4 ± 0.3 and 3.8 ± 2.3 MPs/L at the inlet and from 0.1 ± 0.0 to 1.3 ± 1.0 MPs/L at the outlet. In the sediment, abundance of MPs was generally higher at the inlet, ranging from 736 ± 335 to 3480 ± 4330 MPs/kg dry sediment and decreased to between 19.0 ± 16.4 and 1060 ± 326 MPs/kg dry sediment at the outlet. Although no significant differences were observed in sediment cores at different depth across the five CWs, more MPs were recorded in silt compared to sandy sediment which indicated sediment grain size could be an environmental factor contributing to the distribution of MPs. Polyethylene terephthalate (PET) fibres were the dominant polymer type found in the water samples while polyethylene (PE) and polypropylene (PP) fragments were predominantly recorded in the sediment. While the size of MPs in water varied across the studied CWs, between 51% and 64% of MPs in the sediment were smaller than 300 µm, which raises concerns about the bioavailability of MPs to a wider range of wetland biota and their potential ecotoxicological effects. This study shows that CWs can not only retain MPs in the treated water, but also become sinks accumulating MPs over time.

Keywords: Constructed wetland; Microplastic; Stormwater; Wastewater; Water treatment

1. Introduction

Microplastics (MPs), a type of emerging contaminant smaller than 5 mm in size, have received significant attention because of their widespread presence across many environmental matrices worldwide, including marine environments (Peng et al., 2020), freshwater (Lu et al., 2021), soil (Yang et al., 2021) and even the atmosphere (Zhang et al., 2020b). This widespread occurrence has raised concerns about the bioavailability of MPs to exposed biota. Accumulation of MPs has been documented in a range of aquatic and terrestrial organisms, such as vertebrates, invertebrates and mammals, and birds (Bellasi et al., 2020; de Sa et al., 2018; Kukkola et al., 2021; Ribeiro et al., 2019). In addition, MPs can leach chemical additives (Oliviero et al., 2019) and act as vectors for contaminants, such as metals (Khalid et al., 2021) and organic compounds (Mei et al., 2020).

Due to a diversity of sources, such as textiles, personal care products, packaging, agriculture and household products, MPs enter the environment via several pathways (Peng et al., 2020). Among them, wastewater discharge and stormwater runoff are suggested to be important pathways between land-based MP sources and the aquatic environment (Duis & Coors, 2016; Shruti et al., 2021; Ziajahromi et al., 2016). The discharge of MPs via wastewater treatment plants (WWTPs) has been well documented in the literature (Sun et al., 2019; Ziajahromi et al., 2021). Treatment processes, including primary, secondary and tertiary treatment, can significantly reduce the abundance of MP in wastewater effluent, with a mean removal efficiency of 86% (Liu et al., 2021). The presence of MPs in stormwater has received attention in more recent years. For example, the mean abundance of MPs larger than 80 μm ranged from 3 to 129 MPs/L in Paris (Treilles et al., 2021), while 88 to 289 MPs/L were reported for MPs larger than 25 μm in Mexico (Piñon-Colin et al., 2020). Despite stormwater potentially contributing significant amount of MPs to the environment, a recent study reported that 84% of MPs in stormwater were removed by bioretention cells (Smyth et al., 2021). These results

66 suggest that wastewater and stormwater-based MPs can be mitigated if proper management
67 systems are implemented.

68 Recently, constructed wetlands (CWs) have received attention as a potential treatment option
69 to remove MPs because of their removal efficiency of total suspended solids (Vymazal, 2019).
70 Wang et al. (2020) reported an average removal efficiency of 88% for MPs ranging from 40 to
71 5600 μm by a wastewater treatment CW located in Belgium. Similarly, more than 89% of MPs
72 (20-5000 μm) were removed by two CW systems in China (Zhou et al., 2022). Although the
73 primary objective of CWs is to improve the quality of treated water to protect downstream
74 waters, pollutants, such as heavy metals and organic pollutants are reported to accumulate in
75 the CWs over time (Haberl et al., 2003; Sharley et al., 2017). Therefore, MPs filtered out of
76 treated water are expected to accumulate in CWs and pose a risk to biota dwelling in the CWs
77 because they also provide habitat to a range of fauna, including birds, fish and amphibians
78 (Townsend et al., 2019). The accumulation of pollutants in CWs can therefore conflict with
79 their ecological values. In recent study, MPs in the sediment of a stormwater CW were reported
80 with an average MP abundance of 320 and 595 MPs/kg at the outlet and inlet, respectively, in
81 Australia (Ziajahromi et al., 2020). A previous study reported that the survival, growth and
82 emergence of *Chironomus tepperi* were negatively affected when exposed to MPs with
83 abundance of 500 MPs/kg sediment (Ziajahromi et al., 2018). This suggests that the retention
84 of MPs in CWs may induce adverse effects. However, there is limited information available to
85 understand the abundance of MPs in CW sediment.

86 To provide information about the potential role of CWs in managing MP contamination, four
87 stormwater CWs and one wastewater CW in Southeast Queensland were selected. The aim of
88 the present study was to a) quantify and characterise MPs in water and sediment of the studied
89 CWs and b) investigate the distributions of MPs within CWs by collecting samples at different

90 locations within CWs. In addition, the present study investigated MP abundance in sediment
91 with different depth and granulometry.

92 ## 2. Materials and methods

93 ### 2.1. Sampling

94 Water and sediment core samples from four stormwater constructed wetlands (CW1-CW4)
95 located across the Gold Coast, Australia were collected between June and July 2021. A
96 wastewater constructed wetland (CW5) located in the Sunshine Coast, Australia was sampled
97 in September 2021 (Table S1). Sampling at the stormwater CWs was conducted in dry weather
98 with an antecedent dry period of at least two days. The location of each wetland site is shown
99 in Figure 1. Samples were collected on the same day at each wetland site. To avoid disturbed
100 sediment contributing to the MP abundance in the water phase, water samples were collected
101 before the sediment samples. Both water and sediment samples were collected near the inlet
102 and outlet of each wetland to investigate the distribution and retention of MPs (Figure S1). At
103 each sampling location, water and sediment samples were sampled in triplicate at least 5 m
104 apart.

105 Bulk water samples of 10 L were collected using amber glass bottles from each sampling point
106 at 0 to 5 cm below the water surface, resulting in a total of 30 water samples across the 5 sites.
107 Samples were stored at 4 °C in the laboratory. To monitoring potential MP contamination
108 during sampling, an empty glass bottle (field blank) was left open for the duration of sampling.

109 Sediment cores were collected with a polycarbonate corer (7 cm inner diameter) taken to a
110 maximum depth of 24 cm and were sliced into 2 cm segments using a stainless-steel scraper.
111 The depth of each sediment core was different, ranging from 2 to 24 cm. To facilitate
112 comparison across sites, only sediment cores from the top 8 cm were processed, resulting in a
113 total of 109 sediment core samples across 5 sites.

2.2. Sample processing

To process water samples, the protocol validated by Ziajahromi et al. (2020) was adopted with some modifications. The bulk water sample (10 L) was first filtered through 25 µm stainless-steel filters. The retained particles on the filters were then washed into a 250 mL beaker with 150 mL ultrapure water and covered with a watch glass. To remove non-polymer organic materials in the water, 50 mL of 30% hydrogen peroxide (H_2O_2, Chem-Supply, Australia) was added to the beaker in 10 mL increments every 30 min and left in a shaker incubator (120 rpm) at 50 °C. After 24 h of digestion, samples were transferred to 50 mL centrifuge tubes with ultrapure water. If the solution was more than 50 mL, multiple tubes were used and topped up with ultrapure water to the 50 mL mark. The tubes were manually shaken for 1 min and then centrifuged for 10 min at 3000×*g*. The supernatants were then vacuum filtered through 25 µm stainless-steel filters. If there were visible contents settled at the bottom of the tubes, a density separation was conducted using sodium iodide (NaI, Chem-Supply, Australia) with a density of 1.8 g/cm^3 to increase the extraction rate of MPs. After manually shaking for 1 min, the tubes were centrifuged for 10 min at 3000×*g* and the supernatants were filtered through the same 25 µm filters. Each filter was placed in a glass Petri dish covered with a lid and dried in an oven at 40 °C for microscopy.

Sediment core samples were homogenised using a glass rod and dried in an oven at 50 °C until the water content was fully evaporated. The processing of sediment core samples was based on protocols described by Hurley et al. (2018) and Ziajahromi et al. (2021). Briefly, 50 g of dried sediment core was weighed in a 250 mL beaker and covered with a watch glass. If the dried sediment core was less than 50 g, the whole core sample was used, and the weight recorded. Fenton's reagent was used to remove non-polymer organic materials by adding 20 mL of 0.05 M ferrous sulfate ($FeSO_4$) solution and 20 mL of 30% H_2O_2. Due to the exothermic reaction, the beaker was placed in an ice-water bath to prevent the sample from overheating. The

digestion was continued by adding another 30 mL H_2O_2 in 5 mL increments. The sample was then left on a stir plate at 150 rpm at room temperature for 24 h, then the temperature was increased to 50 °C for another 24 h. After the digestion process was completed, sediment core samples underwent the same separation process and subsequent filtration using 25 µm stainless-steel filters as described for the water samples.

2.3. MP quantification and characterisation

Filters from each sample were inspected under a stereo microscope (Olympus SZX10) equipped with a digital camera. The suspected MPs were counted and classified according to their shape, namely fibre, fragment, granule (bead), film and foam, using the point counting tool in the CellSens Standard image analysis software (Olympus). The categorisation of each shape referred to the physical feature described by Lusher et al. (2020). The size of individual particles was measured using the measuring tool available in the software using the longest dimension of the particle.

The suspected particles were analysed using a PerkinElmer Spotlight 400 Fourier-transform infrared spectroscopy (FTIR) Imaging System with a Mercury-Cadmium-Telluride (MCT) detector cooled in liquid nitrogen, operating in reflectance mode and with a wavenumber resolution of 4 cm^{-1}. A total of 16 scans were collected across a wavenumber range from 4000 to 650 cm^{-1}. Particles larger than 1 mm were individually transferred with tweezers from filters to the diamond crystal of the Attenuated Total Reflectance (ATR) accessory while smaller particles (< 1 mm) were analysed using µFTIR. Spectra were subjected to a library search using the search routine of the Nicolet Omnic 9.2 software using a library set that included the Nicolet polymer, forensics and common materials set in addition to the Hummel polymer library. The search routine produced a score out of 100 for goodness of match. Polymer composition of the particle was automatically accepted if the match score exceeded 80 against reference spectrum. Peaks of spectra with match score between 60 and 80 were further examined because particles

might be subjected to weathering or biofouling. To provide comprehensive information about MPs, all suspected MPs in the water samples and all fibres in sediment samples were analysed by FTIR, while non-fibrous particles with found in sediment were randomly selected for FTIR analysis to avoid only analysing particles with similar visual characteristics (e.g., shape and colour). This means that all suspected MPs in water samples (n = 327) and 50% of suspected particles in sediment (n = 1609) were analysed by FTIR.

2.4. Sediment grain size analysis

To further investigate the relationship between the sediment grain size and the abundance of MPs in the sediment cores, grain size analyses were performed using laser diffraction spectroscopy (Malvern Mastersizer 3000, Malvern Instruments) which detects particles with size range from 0.01 to 2000 μm. For each analysed sediment core, an adequate amount of sediment was added to the dispersion unit filled with deionised water to obtain an obscuration value of between 10 and 20% as recommended in the user manual. For analysis, sediment grain was categorised according to mean particle size, namely clay (<2 μm), silt (2-63 μm) and sand (>63 μm).

2.5. Quality assurance and quality control

To ensure the reliability of the selected processing methods, positive controls were included in every batch of 6 water samples while triplicates of positive controls were performed for sediment core samples for each site. Seven common polymers with different colours, shapes and size ranges were prepared from consumer products using a kitchen grater and scissors (Table S2). Ten particles of each polymer were spiked in clean water and sediment and processed with the same experimental steps from digestion to microscopy.

Negative control samples were processed through all steps of the sample processing workflow to determine any potential background plastic contamination from the laboratory (laboratory

188 blanks) (e.g., contamination from air, operators and used chemicals) as well as during sampling
189 (field blanks) to monitor potential contamination from the atmosphere and the sampling
190 containers (Lu et al., 2021). The control beakers were rinsed with ultrapure water and processed
191 using the same methodology as described above for the water and sediment samples.

192 Additionally, to avoid potential background contamination during the sample analysis process
193 in the laboratory, a series of preventive measures described by Lu et al. (2021) were adopted.
194 All sampling containers, equipment and laboratory glassware were rinsed three times with
195 ultrapure water before use and laboratory glassware was covered with a watch glass. All
196 chemicals were vacuum filtered through nylon filters (Whatman®, pore size: 0.45 µm,
197 diameter: 47 mm) immediately prior to use. Petri dishes were kept closed during microscopy
198 and the lids were only opened if samples or particles needed to be removed. Cotton lab coats
199 and nitrile gloves were worn during the whole process. All process steps were conducted in a
200 fume hood.

201 **2.6. Statistical analysis**

202 Abundance of MPs were reported as MPs/L and MPs/kg dry sediment for water and sediment
203 samples, respectively. Due to the high skewness, MP abundance data of both water and
204 sediment were log-transformed. A Shapiro–Wilk test was then used to test the normality of the
205 data. If normality was satisfied, a parametric test one-way ANOVA was applied to examine
206 the differences between the abundance of MPs across and within sites based on factors such as
207 sampling location and sediment grain size. Otherwise, a non-parametric Kruskal-Wallis test
208 was used. If significant differences were observed, a post hoc test was performed (Tukey test
209 for ANOVA and Dunn test with a Bonferroni correction for Kruskal Wallis). All data were
210 recorded using Microsoft Excel and graphs were produced in GraphPad Prism (v9.3.1),
211 Statistical analyses were conducted using R (v4.1.2) with the significance level set to $\alpha = 0.05$.

3. Results and discussion

3.1. Quality assurance and quality control

Average recovery rates of positive controls were 90% and 82% in water and sediment, respectively (Table S2). Because positive controls are not commonly included in MP studies, there is no standardised threshold to decide whether the processing protocol is deemed to be effective (Lu et al., 2021). A recent review reported that the overall recovery rates reported in water and sediment samples ranged from 67% to 88% (Way et al., 2022). Therefore, our results of recovery rates are comparable to other literature. It should be noted that the lower size fraction of spiked MPs ranged between 150 and 250 μm which could underestimate the abundance of MPs because smaller MPs are more likely to be lost during sample processing.

A total of 12 and 10 fibres were found in the field blanks and laboratory blanks, respectively, for both water and sediment samples (Table S3). However, all the fibres were identified as cellulosic fibres after FTIR analyses. Therefore, no blank correction was performed. None of the particles identified in the sediment matched the polycarbonate material of the core sampling tube, meaning that the coring tube itself did not cause contamination in the present study.

Some of the particles collected in the water (44 particles; 13%) and sediment samples (41 particles; 3%) were identified as non-plastic by FTIR which were all cellulosic fibres. Although some cellulosic fibres, such as rayon and viscose, are considered as MPs in some studies, it is difficult to distinguish natural and regenerated cellulose fibres based on the FTIR spectra. Therefore, cellulosic fibres were excluded from the final count of MPs in the results. The spectra of some analysed particles could not be identified, accounting for 2% (6 particles) and 11% (175 particles) of particles in water and sediment samples, respectively. These particles were not excluded from the final MP count if their shapes and colours were anthropogenic as outlined in protocols described by Lusher et al. (2020), but were hereafter identified as

236 "unknown MP". The group of "unknown MP" also contained particles with spectra identified
237 as plasticizer. Black fragments found in the present study were not considered as MPs if the
238 chemical composition was not confirmed. Many of the black fragments have a rubbery texture
239 when squeezed with tweezers and may originate from carbon black loaded styrene-butadiene
240 rubber (SBR) (Figure S2). However, the analysis of black fragments by direct-on-filter
241 measurements by reflectance μFTIR is hindered by strong absorption by carbon (Ziajahromi
242 et al., 2020).

243 **3.2. Abundance of MPs in water and sediment**

244 MPs were found in 29 out of 30 collected water samples with the abundance ranging from 0.4
245 ± 0.3 to 1.2 ± 0.4 MPs/L in inlet water and 0.1 ± 0.0 to 0.5 ± 0.4 MPs/L in outlet water samples
246 from the stormwater CWs (Figure 2, CW1-CW4). The abundance of MPs was significantly
247 higher in water samples at the inlet (ANOVA, $F(4, 10) = 4.621$, $p < 0.05$) of the wastewater
248 CW (Figure 2, CW5), with an average abundance of 3.8 ± 2.3 MPs/L. Post hoc Tukey test
249 revealed that significant differences were observed between CW2 and CW5 ($p < 0.05$) and
250 CW4 and CW5 ($p < 0.05$) (Figure 2). However, the difference was not significant at the outlet
251 of CW5 compared to the four stormwater CWs (ANOVA, $F(4, 9) = 2.360$, $p > 0.05$) which
252 had an average abundance of 1.3 ± 1.0 MPs/L. The higher MP abundance at the inlet of CW5
253 may result from the constant input of wastewater-derived MPs from the upstream WWTP,
254 whereas stormwater CWs normally only receive influent during rain events.

255 Within each CW, there were no significant differences between the inlet and outlet (ANOVA,
256 $p > 0.05$), despite lower MP abundance being detected at the outlet. Our results are in a similar
257 range as those reported by Ziajahromi et al. (2020), which found 0.9 and 4.0 MPs/L at the inlet
258 and outlet of a stormwater CW, respectively. While the opposite trend to the current study was
259 reported, with the MP abundance higher at the outlet than the inlet, it should be noted that
260 Ziajahromi et al. (2020) collected water samples after a heavy rain event, which may have

contributed to the higher MP abundance in the outlet. Liu et al. (2019a) investigated the load of MPs in seven stormwater ponds during dry weather in Denmark and reported abundance of MPs ranging from 0.5 to 22.9 MPs/L, while Olesen et al. (2019) detected MP abundance as high as 250 MPs/L in a stormwater retention pond in Denmark. The higher MP abundance in the two studies may result from the smaller filter size used, 10 µm, compared to 25 µm used in the current study. It is suggested that the reported MP abundance can be affected by the mesh size used for water sampling (Hidalgo-Ruz et al., 2012). The result of a recent review also showed a relationship between filter size and the abundance of MP with studies using smaller mesh sizes often reporting more MPs (Lu et al., 2021).

MPs were detected in 105 out of 109 sediment cores. Overall, the abundance of MPs in sediment were not significantly different between stormwater and wastewater CWs, which suggested that stormwater runoff could be a significant source of MPs in aquatic environments if the runoff is not treated properly. The mean MP abundance in sediment samples at the inlet of stormwater CWs ranged from 736 ± 335 (CW2) to 2610 ± 1950 MPs/kg dry sediment (CW3) (Figure 3). In contrast to the water phase, there were no significant differences between inlet sediments of wastewater CW and stormwater CWs (ANOVA, $F(4, 52) = 2.193$, $p > 0.05$) with a mean MP abundance of 3480 ± 4330 MPs/kg in the wastewater CW. Within each wetland, a significantly more MPs was recorded in the samples collected from the inlet compared to the outlet except for CW1 (ANOVA, $F(1, 22) = 1.193$, $df = 1$, $p > 0.05$), with the MP abundance in the outlet sediment ranging from 19.0 ± 16.4 (CW5) to 1060 ± 326 MPs/kg dry sediment (CW1). A similar trend was reported in a recent study by Ziajahromi et al. (2020), with the average MP abundance in sediment decreasing from 595 MPs/kg at the inlet to 320 MPs/kg at the outlet of a stormwater treatment CW. The different distribution patterns of MPs in the sediment may be affected by CW characteristics, such as the type of wetland, flow distance between inlet and outlet, and macrophyte zone etc. Detecting more MPs in the outlet sediment

samples at CW1 is likely due to shorter flow distance between inlet and outlet compared to other studied CWs (Table S1).

Despite a lack of information about MP abundance in the sediment of wastewater treatment CWs, recent studies on stormwater CW and other stormwater treatment systems, such as retention ponds and detention reservoirs could provide some information about the retention of MPs. In Denmark, MP abundance ranged from 1,511 to 127,986 MPs/kg in 7 stormwater retention ponds (Liu et al., 2019b) while the MP abundance was as high as 950,000 MPs/kg in another Danish stormwater retention pond (Olesen et al., 2019). Moruzzi et al. (2020) investigated a stormwater detention reservoir in Brazil and reported an average MP abundance of 57,542 MPs/kg. Although our results were not as high as these stormwater studies, the average MP abundance at the five CWs (1050 MPs/kg) was two times higher than the abundance of MPs reported by Ziajahromi et al. (2020) and higher than nearly 80% of reported freshwater sediment samples (Lu et al., 2021). There are several factors that need to be considered when comparing the abundance of MPs among studies. First, the present study used 25 μm as the lower size limit compared to 10 μm used by Liu et al. (2019b) and Olesen et al. (2019). As mentioned above, MP abundance is expected to increase when smaller filter mesh sizes are used to isolate MPs. Second, the abundance of MPs detected is related to the service time of the studied CW or stormwater treatment system. For instance, the floating stormwater CW sampled by Ziajahromi et al. (2020) was installed in 2016, which could explain the lower MP abundance detected by the authors. Conversely, the high abundance detected by Olesen et al. (2019) could be attributed to the age of the stormwater retention pond, which is reported to be more than 30 years old, leading to the accumulation of more MPs. Finally, MP abundance can be associated with the type of land use and population density within the catchment of the study sites (Townsend et al., 2019). The present study did not investigate the correlation between MP abundance and land use within the wetland catchment due to the lack of

311 information about the catchment area and stormwater drainage systems of each CW. However,
312 the four stormwater CWs are located in proximity to residential areas while the retention pond
313 investigated in Olesen et al. (2019) had a catchment area categorised as light industry with
314 roads with semi-heavy traffic.

315 Vertically, there was no significant difference between MP abundance and the depth of
316 sediment cores at the inlet (ANOVA, $p > 0.05$) and outlet (ANOVA, $p > 0.05$) at any of the
317 sites (Figure S3). Studies investigating MPs in sediment cores have reported a temporal trend
318 where less MP were recorded with increasing depth of the sediment core in Tokyo Bay
319 (Matsuguma et al., 2017), an urban estuary in Australia (Willis et al., 2017) and an urban lake
320 in United Kingdom (Turner et al., 2019). These results found that the abundance of MPs in the
321 studied sediment cores proliferated after 1950s which coincided with the increasing production
322 of plastics. Compared to the previous studies, the four stormwater CWs investigated in the
323 current study are comparatively new, having been constructed between 2000 and 2010 while
324 the wastewater CW was constructed back in 1995 with some periodic augmentations since then.
325 A likely explanation of the vertical trend in the present study is that the accumulation of MPs
326 in CWs is affected by the variation in frequency and intensity of runoff that the wetlands receive.

327 Apart from MP distribution in the sediment, the present study also investigated whether
328 sediment grain size could be a factor affecting the abundance of MPs. Overall, the sediment
329 cores were mainly characterised as silt (89%), with only 12 cores characterised as sand (11%)
330 which were sampled from the inlet of CW1, CW3 and CW4, respectively (Table S4).
331 Significance differences were found at the three sites between the grain size of sediment and
332 MP abundance ($p < 0.05$) with more MPs recorded in the sediment with a finer grain size
333 (Figure S4). Similar results were reported by other studies investigating the abundance of MPs
334 in freshwater sediment with different grain size. For example, Corcoran et al. (2020) reported
335 significantly higher MP abundance in very fine sand samples than in fine + medium sand.

Likewise, a recent study found that the percentage of silt in sediment samples was positively correlated with the abundance of MPs (Cera et al., 2022). In their study, Enders et al. (2019) found a strong correlation between the sediment grain size and high density of MPs with size >500 μm and suggested the former could be an important proxy for the abundance of MPs. In addition, Vermeiren et al. (2021) observed an exponential increase in MP abundance with decreasing grain size. Despite the mechanisms behind the deposition and distribution of MPs in sediment being still poorly understood, the results suggest that sediment granulometry could be a critical factor affecting the distribution of MPs in the sediment and need to be taken into account when comparing MP abundance.

The primary function of CWs is to reduce the concentration of solids, nutrients, and pollutants in the influent prior to discharge to the receiving environment. Therefore, CWs can become sinks for pollutants which can accumulate over time. Recent studies reported nearly 90% removal efficiency of MPs in wastewater CWs, which shed lights on the application of CWs in mitigating MPs contamination in waterways (Wang et al., 2020; Zhou et al., 2022). In other words, MPs are retained in CWs and accumulate over time. Because CWs contain a community of bacteria and other microorganisms to facilitate the microbial processes in the wetlands (Kadlec et al., 2000), the adsorption of these microorganisms on MPs could form a biofilm. The formation of a biofilm on the surface of MPs is reported to affect their physical and chemical properties, such as weight, surface morphology, density and hardness, and therefore affect the interaction between MPs and exposed organisms (Luo et al., 2022; Rummel et al., 2017). In addition, pollutants, including heavy metal and organic pollutants removed from the treated wastewater and stormwater by CWs can adsorb to MPs and pose risks to wetland dwelling organisms (Khalid et al., 2021; Mei et al., 2020). MPs in sediment may also be resuspended when the CW receives large influent volumes during intense flood events.

Therefore, CWs could act as secondary sources, with the high abundance of MPs in sediment resuspended in the water and released into downstream waterbodies.

3.3. Characteristics of MPs

3.3.1. Shapes of MPs

Fibres and fragments were detected in the water samples with fibres predominant across all sites except CW3, where fragments accounted for more than 60% in both the inlet and outlet (Figure 4a). Conversely, fragments ranged from 70% to 87% and 50% to 86% of MPs in the sediment samples collected from the inlet and outlet across all sites, respectively (Figure 4b). The dominance of fibres and fragments is consistent with the findings reported in water and sediment across freshwater environments (Lu et al., 2021). Apart from fibres and fragments, films and foams were also found in sediment samples (Figure S5).

The shapes of MPs found in the environment are important indicators of the sources. Fibrous MPs, which mainly originate from textiles, were reported as a major shape of MPs detected in wastewater (Talvitie et al., 2017; Ziajahromi et al., 2017; Ziajahromi et al., 2021). Therefore, the high abundance of fibres detected in the water phase of the wastewater CW is unsurprising. Indeed, a significantly higher number of fibres in water samples was observed in the wastewater CW compared to its stormwater counterpart in the present study (ANOVA, $F(1, 23) = 10.495$. $p < 0.05$). Fibrous MPs in stormwater CWs, on the other hand, are most likely contributed by surface runoff or stormwater drainage because these sites are proximal to residential areas. Another potential source of fibres found in water was the deposition from the atmosphere (Dris et al., 2016). A recent study investigating MPs in stormwater control measures reported that atmospheric deposited or wind-blown MPs contributed to the accumulation (Koutnik et al., 2022). Fragments, which were reported in more than 80% of the reviewed freshwater studies, generally result from the breakdown of large plastic items, such

as food packaging and bottles (Lu et al., 2021). Because CWs remove pollutants through physical and biological processes, plastics and MPs retained in CWs are expected to break into smaller particles over time. The dominant MP shapes in water and sediment in the present study show an opposite trend compared to a previous study where fragments were predominantly found in water and fibres accounted for the majority of MPs in sediment in a stormwater treating CW (Ziajahromi et al., 2020). However, the differences may be affected by the sources of stormwater that the constructed CW received. Interestingly, no granular shaped MPs (commonly referred to as granules or beads) were detected in the present study. Granular MPs generally originate from personal care products, cosmetics and industrial raw products which are the most commonly used shape of MPs in toxicity studies (Ockenden et al., 2021). The prevalence of fragmented MPs indicated that plastic litter degradation, also known as secondary MPs, is a significant source of MPs in urban environments and therefore should be considered for future toxicity studies.

3.3.2. Chemical composition of MPs

With respective to chemical composition, 12 polymers were identified in the water samples, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyamide (PA), polyvinyl alcohol (PVA), acrylic, polyacrylonitrile (PAN), polyurethane (PU), alkyd, and acrylonitrile butadiene styrene (ABS). Compared with water samples, more polymer types were identified in the sediment samples. In addition to the 12 polymers detected in water, PP-PE copolymer (PP-PE), phenol resin, epoxy resin, ethylene vinyl acetate (EVA), phenoxy resin, styrene acrylonitrile (SAN) and polybutylene terephthalate (PBT) were also detected in sediment. To facilitate comparison between the water and sediment samples, polymers, including “Unknown MP” and polymers other than PE, PP, PS, PET, PVC, PA and acrylic were grouped as “Others” which were

equivalent to 8% and 15% of MPs found in water and sediment samples, respectively. FTIR spectra of some polymers found in current studies are presented in Figure S6-S10.

PET was the most abundant polymer in the water samples accounting for 66% of the MPs, followed by PE (10%) and PP (9%) (Figure 5a). The high proportion of PET was mainly due to the predominance of fibres in the water, especially at the wastewater CW (Figure S11). PP, PE and PP-PE mainly in the form of fragments, on the other hand, were dominant in the sediments which comprised of 64% of the MPs (Figure 5b). The potential sources of different polymers are associated with their shapes, which are discussed in Section 3.3.1. Although the density of PET (1.37-1.45 g/cm^3) is higher than water, which means it is more likely to settle at the bottom of the water phase and eventually in sediment, the prevalence of PET in water samples were also reported in other studies (Alfonso et al., 2020; Huang et al., 2020; Migwi et al., 2020; Nan et al., 2020; Oni et al., 2020). Therefore, density cannot be considered as the sole factor contributing to the distribution of MP polymers in the environment. The prevalence of PP and PE in sediment was also reported in other studies (Eo et al., 2019; Klein et al., 2015; Olesen et al., 2019; Ta et al., 2020; Yuan et al., 2019; Zhang et al., 2020a; Zhang et al., 2019). Although PP and PE are plastics with high demands globally due to their versatile applications in food packaging, container, houseware and automotive parts (PlasticsEurope, 2021), their densities are lower than water and hence are more likely to float in the water phase. A possible explanation could be the effects of biofouling which increase the density of MPs and therefore settle in the sediment over time (Eo et al., 2019).

3.3.3. Size distribution of MPs

The size of MPs found in the present study ranged from 25 to 5000 µm in both the water and sediment samples, with the size distribution of the detected MPs across the five CWs shown in Figure 6. Between 14% (CW4) and 51% (CW5) of MPs found in water samples were smaller than 300 µm (Table S5). In sediment samples, on the other hand, between 51% (CW4) and

64% (CW2) of MPs were smaller than 300 μm across all five CWs (Table S6). Because WWTPs can remove large particles during the treatment process (Liu et al., 2021), the sizes of MPs in wastewater effluent were generally smaller than the influent, with an average of 90% of MPs smaller than 500 μm in effluent (Sun et al., 2019). Untreated stormwater received by the stormwater CWs resulted in a wider variation of size. Despite the difficulty in providing a comprehensive comparison between studies because of the lack of standardised methodology and reporting size binning, the abundance of MPs is expected to increase with decreasing sizes (Lu et al., 2021). In surface water from the Yellow River in China, MPs in the size range of 50-100 μm and 100-200 μm accounted for 52% and 35% of the total MPs and decreased to 8% and 5% in the range of 200-500 μm and 500-5000 μm (Han et al., 2020). Similarly, the decreasing trend were observed in Saudi Arabian lakes (Pico et al., 2020). Liu et al. (2019b) reported nearly 95% of MPs in the sediment of 7 stormwater retention ponds were smaller than 250 μm with 51% and 44% of MPs were in the range of 10-50 μm and 50-250 μm, respectively.

Concerns about smaller MPs were raised not only because of increasing bioavailability, but also potential toxicity (Lehtiniemi et al., 2018). It was suggested that smaller MPs are more likely to be retained and therefore settle in the sediment compared to the larger ones (Vermeiren et al., 2021). In addition, smaller MPs have larger surface areas, which increases the chances of sorption of other pollutants and microorganisms in the environment (Han et al., 2020; Luo et al., 2022). Recently, An et al. (2021) reported that higher number of ingested MP as well as higher mortality rates were observed in *Daphnia magna* after exposure to smaller particles sizes (mean size of 17 μm) compared to larger particle sizes (mean size of 34 μm).

4. Conclusion

This study investigated the abundance and distribution of MPs in CWs. The results illustrate that stormwater is a critical pathway transporting MPs, possibly as important as wastewater. Generally, less MPs were recorded at the outlet water sample. In addition, more MPs were

458 observed in the CW sediment samples compared to sediment from other freshwater
459 environments, which suggests that CWs retain significant numbers of MPs over time. Overall,
460 MPs smaller than 300 μm were predominant across the different sites.

461 While this study represents a snapshot in time, the results demonstrate that these engineered
462 systems could retain MPs and eventually act as a sink of MPs. Further study is required to
463 understand if these stormwater management measures can effectively reduce MP transport to
464 downstream environments by investigating the removal of MPs in stormwater CWs under
465 different rain events (particularly the risk of MPs being resuspended and released during high
466 flow events) and provide long-term monitoring information about the potential roles of CWs
467 in mitigating MP contamination in urban areas.

468 **CRediT authorship contribution statement**

469 Hsuan-Cheng Lu: Conceptualization, Methodology, Investigation, Visualization, Formal
470 analysis, Writing – Original Draft

471 Shima Ziajahromi: Conceptualization, Writing – Review & Editing

472 Ashley Locke: Formal analysis, Resources, Writing – Review & Editing

473 Peta A. Neale: Conceptualization, Writing – Review & Editing

474 Frederic D.L. Leusch: Conceptualization, Writing – Review & Editing

475 **Acknowledgements**

476 Hsuan-Cheng Lu is supported by a Griffith University Postgraduate Research Scholarship
477 awarded by Griffith University and a Biosis Ecology Scholarship awarded by Biosis Pty Ltd.
478 This work was enabled by use of the Central Analytical Research Facility (CARF) at the
479 Queensland University of Technology (QUT). We thank Nikol Slynkova and Ryan Wisbey for
480 assistance with sample collection.

Reference

Alfonso, M. B., Scordo, F., Seitz, C., Manstretta, G. M. M., Ronda, A. C., Arias, A. H., Tomba, J. P., Silva, L. I., Perillo, G. M. E., & Piccolo, M. C. (2020). First evidence of microplastics in nine lakes across Patagonia (South America). *Science of the Total Environment, 733*, 8. doi:10.1016/j.scitotenv.2020.139385

An, D., Na, J., Song, J., & Jung, J. (2021). Size-dependent chronic toxicity of fragmented polyethylene microplastics to Daphnia magna. *Chemosphere, 271*. doi:10.1016/j.chemosphere.2021.129591

Bellasi, A., Binda, G., Pozzi, A., Galafassi, S., Volta, P., & Bettinetti, R. (2020). Microplastic contamination in freshwater environments: A review, focusing on interactions with sediments and benthic organisms. *Environments, 7*(4). doi:10.3390/environments7040030

Cera, A., Pierdomenico, M., Sodo, A., & Scalici, M. (2022). Spatial distribution of microplastics in volcanic lake water and sediments: Relationships with depth and sediment grain size. *Science of the Total Environment, 829*. doi:10.1016/j.scitotenv.2022.154659

Corcoran, P. L., Belontz, S. L., Ryan, K., & Walzak, M. J. (2020). Factors controlling the distribution of microplastic particles in benthic sediment of the Thames River, Canada. *Environmental Science & Technology, 54*(2), 818-825. doi:10.1021/acs.est.9b04896

de Sa, L. C., Oliveira, M., Ribeiro, F., Rocha, T. L., & Futter, M. N. (2018). Studies of the effects of microplastics on aquatic organisms: What do we know and where should we focus our efforts in the future? *Science of the Total Environment, 645*, 1029-1039. doi:10.1016/j.scitotenv.2018.07.207

Dris, R., Gasperi, J., Saad, M., Mirande, C., & Tassin, B. (2016). Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? *Marine Pollution Bulletin, 104*(1-2), 290-293. doi:10.1016/j.marpolbul.2016.01.006

Duis, K., & Coors, A. (2016). Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environmental Sciences Europe, 28*. doi:10.1186/s12302-015-0069-y

Enders, K., Käppler, A., Biniasch, O., Feldens, P., Stollberg, N., Lange, X., Fischer, D., Eichhorn, K. J., Pollehne, F., Oberbeckmann, S., & Labrenz, M. (2019). Tracing microplastics in aquatic environments based on sediment analogies. *Scientific Reports, 9*(1). doi:10.1038/s41598-019-50508-2

Eo, S., Hong, S. H., Song, Y. K., Han, G. M., & Shim, W. J. (2019). Spatiotemporal distribution and annual load of microplastics in the Nakdong River, South Korea. *Water Research, 160*, 228-237. doi:10.1016/j.watres.2019.05.053

Haberl, R., Grego, S., Langergraber, G., Kadlec, R. H., Cicalini, A. R., Dias, S. M., Novais, J. M., Aubert, S., Gerth, A., Thomas, H., & Hebner, A. (2003). Constructed wetlands for the treatment of organic pollutants. *Journal of Soils and Sediments, 3*(2), 109-124. doi:10.1007/BF02991077

Han, M., Niu, X., Tang, M., Zhang, B. T., Wang, G., Yue, W., Kong, X., & Zhu, J. (2020). Distribution of microplastics in surface water of the lower Yellow River near estuary. *Science of the Total Environment, 707*. doi:10.1016/j.scitotenv.2019.135601

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: A review of the methods used for identification and quantification. *Environmental Science & Technology, 46*(6), 3060-3075. doi:10.1021/es2031505

Huang, Y. L., Tian, M., Jin, F., Chen, M. Y., Liu, Z. G., He, S. Q., Li, F. X., Yang, L. Y., Fang, C., & Mu, J. L. (2020). Coupled effects of urbanization level and dam on microplastics

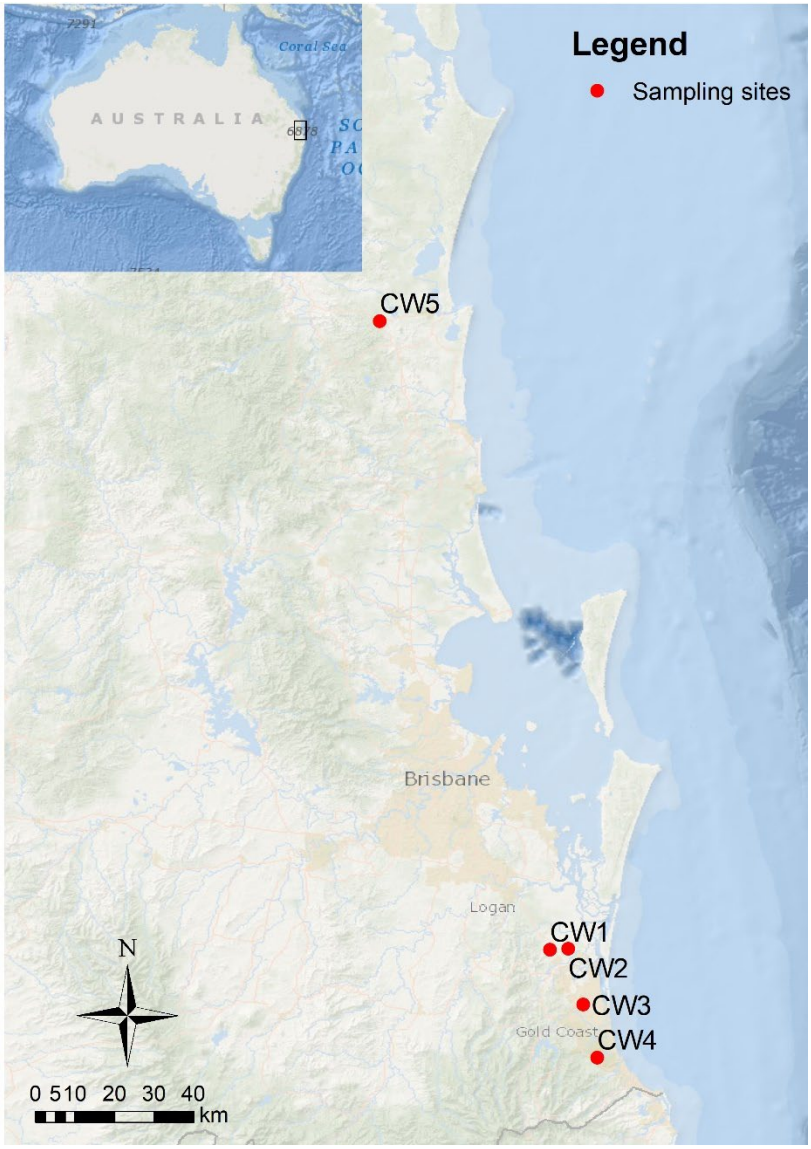
529 in surface waters in a coastal watershed of Southeast China. *Marine Pollution Bulletin,*
530 *154*. doi:10.1016/j.marpolbul.2020.111089
531 Hurley, R. R., Lusher, A. L., Olsen, M., & Nizzetto, L. (2018). Validation of a method for
532 extracting microplastics from complex, organic-rich, environmental matrices.
533 *Environmental Science & Technology, 52*(13), 7409-7417.
534 doi:10.1021/acs.est.8b01517
535 Kadlec, R. H., Knoght, R. L., Vymazal, J., Brix, H., Cooper, P., & Haberl, R. (2000).
536 *Constructed wetlands for pollution control: processes, performance, design and*
537 *operation*: IWA Publishing.
538 Khalid, N., Aqeel, M., Noman, A., Khan, S. M., & Akhter, N. (2021). Interactions and effects
539 of microplastics with heavy metals in aquatic and terrestrial environments.
540 *Environmental Pollution, 290*. doi:10.1016/j.envpol.2021.118104
541 Klein, S., Worch, E., & Knepper, T. P. (2015). Occurrence and spatial distribution of
542 microplastics in river shore sediments of the Rhine-Main area in Germany.
543 *Environmental Science & Technology, 49*(10), 6070-6076.
544 doi:10.1021/acs.est.5b00492
545 Koutnik, V. S., Leonard, J., Glasman, J. B., Brar, J., Koydemir, H. C., Novoselov, A., Bertel,
546 R., Tseng, D., Ozcan, A., Ravi, S., & Mohanty, S. K. (2022). Microplastics retained in
547 stormwater control measures: Where do they come from and where do they go? *Water*
548 *Research, 210*. doi:10.1016/j.watres.2021.118008
549 Kukkola, A., Krause, S., Lynch, I., Sambrook Smith, G. H., & Nel, H. (2021). Nano and
550 microplastic interactions with freshwater biota – Current knowledge, challenges and
551 future solutions. *Environment International, 152*. doi:10.1016/j.envint.2021.106504
552 Lehtiniemi, M., Hartikainen, S., Näkki, P., Engström-Öst, J., Koistinen, A., & Setälä, O. (2018).
553 Size matters more than shape: Ingestion of primary and secondary microplastics by
554 small predators. *Food Webs, 17*. doi:10.1016/j.fooweb.2018.e00097
555 Liu, F., Olesen, K. B., Borregaard, A. R., & Vollertsen, J. (2019a). Microplastics in urban and
556 highway stormwater retention ponds. *Science of the Total Environment, 671*, 992-1000.
557 doi:10.1016/j.scitotenv.2019.03.416
558 Liu, F., Vianello, A., & Vollertsen, J. (2019b). Retention of microplastics in sediments of urban
559 and highway stormwater retention ponds. *Environmental Pollution, 255*, 8.
560 doi:10.1016/j.envpol.2019.113335
561 Liu, W., Zhang, J., Liu, H., Guo, X., Zhang, X., Yao, X., Cao, Z., & Zhang, T. (2021). A review
562 of the removal of microplastics in global wastewater treatment plants: Characteristics
563 and mechanisms. *Environment International, 146*. doi:10.1016/j.envint.2020.106277
564 Lu, H. C., Ziajahromi, S., Neale, P. A., & Leusch, F. D. L. (2021). A systematic review of
565 freshwater microplastics in water and sediments: Recommendations for harmonisation
566 to enhance future study comparisons. *Science of the Total Environment, 781*.
567 doi:10.1016/j.scitotenv.2021.146693
568 Luo, H., Liu, C., He, D., Xu, J., Sun, J., Li, J., & Pan, X. (2022). Environmental behaviors of
569 microplastics in aquatic systems: A systematic review on degradation, adsorption,
570 toxicity and biofilm under aging conditions. *Journal of Hazardous Materials, 423*.
571 doi:10.1016/j.jhazmat.2021.126915
572 Lusher, A. L., Bråte, I. L. N., Munno, K., Hurley, R. R., & Welden, N. A. (2020). Is it or isn't
573 it: The importance of visual classification in microplastic characterization. *Applied*
574 *Spectroscopy, 74*(9), 1139-1153. doi:10.1177/0003702820930733
575 Matsuguma, Y., Takada, H., Kumata, H., Kanke, H., Sakurai, S., Suzuki, T., Itoh, M., Okazaki,
576 Y., Boonyatumanond, R., Zakaria, M. P., Weerts, S., & Newman, B. (2017).
577 Microplastics in sediment cores from Asia and Africa as indicators of temporal trends

578 in plastic pollution. *Archives of Environmental Contamination and Toxicology, 73*(2),
579 230-239. doi:10.1007/s00244-017-0414-9
580 Mei, W., Chen, G., Bao, J., Song, M., Li, Y., & Luo, C. (2020). Interactions between
581 microplastics and organic compounds in aquatic environments: A mini review. *Science*
582 *of the Total Environment, 736*. doi:10.1016/j.scitotenv.2020.139472
583 Migwi, F. K., Ogunah, J. A., & Kiratu, J. M. (2020). Occurrence and spatial distribution of
584 microplastics in the surface waters of Lake Naivasha, Kenya. *Environmental*
585 *Toxicology and Chemistry, 39*(4), 765-774. doi:10.1002/etc.4677
586 Moruzzi, R. B., Speranza, L. G., Conceicao, F. T., Martins, S. T. D., Busquets, R., & Campos,
587 L. C. (2020). Stormwater detention reservoirs: An opportunity for monitoring and a
588 potential site to prevent the spread of urban microplastics. *Water, 12*(7).
589 doi:10.3390/w12071994
590 Nan, B., Su, L., Kellar, C., Craig, N. J., Keough, M. J., & Pettigrove, V. (2020). Identification
591 of microplastics in surface water and Australian freshwater shrimp Paratya australiensis
592 in Victoria, Australia. *Environmental Pollution, 259*.
593 doi:10.1016/j.envpol.2019.113865
594 Ockenden, A., Tremblay, L. A., Dikareva, N., & Simon, K. S. (2021). Towards more
595 ecologically relevant investigations of the impacts of microplastic pollution in
596 freshwater ecosystems. *Science of the Total Environment, 792*.
597 doi:10.1016/j.scitotenv.2021.148507
598 Olesen, K. B., Stephansen, D. A., van Alst, N., & Vollertsen, J. (2019). Microplastics in a
599 stormwater pond. *Water, 11*(7), 15. doi:10.3390/w11071466
600 Oliviero, M., Tato, T., Schiavo, S., Fernández, V., Manzo, S., & Beiras, R. (2019). Leachates
601 of micronized plastic toys provoke embryotoxic effects upon sea urchin Paracentrotus
602 lividus. *Environmental Pollution, 247*, 706-715. doi:10.1016/j.envpol.2019.01.098
603 Oni, B. A., Ayeni, A. O., Agboola, O., Oguntade, T., & Obanla, O. (2020). Comparing
604 microplastics contaminants in (dry and raining) seasons for Ox-Bow Lake in Yenagoa,
605 Nigeria. *Ecotoxicology and Environmental Safety, 198*.
606 doi:10.1016/j.ecoenv.2020.110656
607 Peng, L., Fu, D., Qi, H., Lan, C. Q., Yu, H., & Ge, C. (2020). Micro- and nano-plastics in
608 marine environment: Source, distribution and threats — A review. *Science of the Total*
609 *Environment, 698*. doi:10.1016/j.scitotenv.2019.134254
610 Pico, Y., Alvarez-Ruiz, R., Alfarhan, A. H., El-Sheikh, M. A., Alshahrani, H. O., & Barcelo,
611 D. (2020). Pharmaceuticals, pesticides, personal care products and microplastics
612 contamination assessment of Al-Hassa irrigation network (Saudi Arabia) and its
613 shallow lakes. *Science of the Total Environment, 701*, 11.
614 doi:10.1016/j.scitotenv.2019.135021
615 Piñon-Colin, T. D., Rodriguez-Jimenez, R., Rogel-Hernandez, E., Alvarez-Andrade, A., &
616 Wakida, F. T. (2020). Microplastics in stormwater runoff in a semiarid region, Tijuana,
617 Mexico. *Science of the Total Environment, 704*. doi:10.1016/j.scitotenv.2019.135411
618 PlasticsEurope. (2021). *Plastics - the Facts 2021*. Retrieved from
619 https://plasticseurope.org/knowledge-hub/plastics-the-facts-2021/
620 Ribeiro, F., O'Brien, J. W., Galloway, T., & Thomas, K. V. (2019). Accumulation and fate of
621 nano- and micro-plastics and associated contaminants in organisms. *TrAC - Trends in*
622 *Analytical Chemistry, 111*, 139-147. doi:10.1016/j.trac.2018.12.010
623 Rummel, C. D., Jahnke, A., Gorokhova, E., Kuhnel, D., & Schmitt-Jansen, M. (2017). Impacts
624 of biofilm formation on the fate and potential effects of microplastic in the aquatic
625 environment. *Environmental Science & Technology Letters, 4*(7), 258-267.
626 doi:10.1021/acs.estlett.7b00164

627 Sharley, D. J., Sharp, S. M., Marshall, S., Jeppe, K., & Pettigrove, V. J. (2017). Linking urban
628 land use to pollutants in constructed wetlands: Implications for stormwater and urban
629 planning. *Landscape and Urban Planning, 162*, 80-91.
630 doi:10.1016/j.landurbplan.2016.12.016
631 Shruti, V. C., Pérez-Guevara, F., Elizalde-Martínez, I., & Kutralam-Muniasamy, G. (2021).
632 Current trends and analytical methods for evaluation of microplastics in stormwater.
633 *Trends in Environmental Analytical Chemistry, 30*. doi:10.1016/j.teac.2021.e00123
634 Smyth, K., Drake, J., Li, Y. R., Rochman, C., Van Seters, T., & Passeport, E. (2021).
635 Bioretention cells remove microplastics from urban stormwater. *Water Research, 191*.
636 doi:10.1016/j.watres.2020.116785
637 Sun, J., Dai, X., Wang, Q., van Loosdrecht, M. C. M., & Ni, B. J. (2019). Microplastics in
638 wastewater treatment plants: Detection, occurrence and removal. *Water Research, 152*,
639 21-37. doi:10.1016/j.watres.2018.12.050
640 Ta, A. T., Babel, S., & Haarstick, A. (2020). Microplastics contamination in a high population
641 density area of the Chao Phraya River, Bangkok. *Journal of Engineering and*
642 *Technological Sciences, 52*(4), 534-545. doi:10.5614/j.eng.technol.sci.2020.52.4.6
643 Talvitie, J., Mikola, A., Koistinen, A., & Setälä, O. (2017). Solutions to microplastic pollution
644 – Removal of microplastics from wastewater effluent with advanced wastewater
645 treatment technologies. *Water Research, 123*, 401-407.
646 doi:10.1016/j.watres.2017.07.005
647 Townsend, K. R., Lu, H. C., Sharley, D. J., & Pettigrove, V. (2019). Associations between
648 microplastic pollution and land use in urban wetland sediments. *Environmental Science*
649 *and Pollution Research, 26*(22), 22551-22561. doi:10.1007/s11356-019-04885-w
650 Treilles, R., Gasperi, J., Gallard, A., Saad, M., Dris, R., Partibane, C., Breton, J., & Tassin, B.
651 (2021). Microplastics and microfibers in urban runoff from a suburban catchment of
652 Greater Paris. *Environmental Pollution, 287*. doi:10.1016/j.envpol.2021.117352
653 Turner, S., Horton, A. A., Rose, N. L., & Hall, C. (2019). A temporal sediment record of
654 microplastics in an urban lake, London, UK. *Journal of Paleolimnology, 61*(4), 449-
655 462. doi:10.1007/s10933-019-00071-7
656 Vermeiren, P., Lercari, D., Muñoz, C. C., Ikejima, K., Celentano, E., Jorge-Romero, G., &
657 Defeo, O. (2021). Sediment grain size determines microplastic exposure landscapes for
658 sandy beach macroinfauna. *Environmental Pollution, 286*.
659 doi:10.1016/j.envpol.2021.117308
660 Vymazal, J. (2019). Is removal of organics and suspended solids in horizontal sub-surface flow
661 constructed wetlands sustainable for twenty and more years? *Chemical Engineering*
662 *Journal, 378*. doi:10.1016/j.cej.2019.122117
663 Wang, Q., Hernández-Crespo, C., Santoni, M., Van Hulle, S., & Rousseau, D. P. L. (2020).
664 Horizontal subsurface flow constructed wetlands as tertiary treatment: Can they be an
665 efficient barrier for microplastics pollution? *Science of the Total Environment, 721*.
666 doi:10.1016/j.scitotenv.2020.137785
667 Way, C., Hudson, M. D., Williams, I. D., & Langley, G. J. (2022). Evidence of underestimation
668 in microplastic research: A meta-analysis of recovery rate studies. *Science of the Total*
669 *Environment, 805*. doi:10.1016/j.scitotenv.2021.150227
670 Willis, K. A., Eriksen, R., Wilcox, C., & Hardesty, B. D. (2017). Microplastic distribution at
671 different sediment depths in an urban estuary. *Frontiers in Marine Science, 4*(DEC).
672 doi:10.3389/fmars.2017.00419
673 Yang, L., Zhang, Y. L., Kang, S. C., Wang, Z. Q., & Wu, C. X. (2021). Microplastics in soil:
674 A review on methods, occurrence, sources, and potential risk. *Science of the Total*
675 *Environment, 780*. doi:10.1016/j.scitotenv.2021.146546

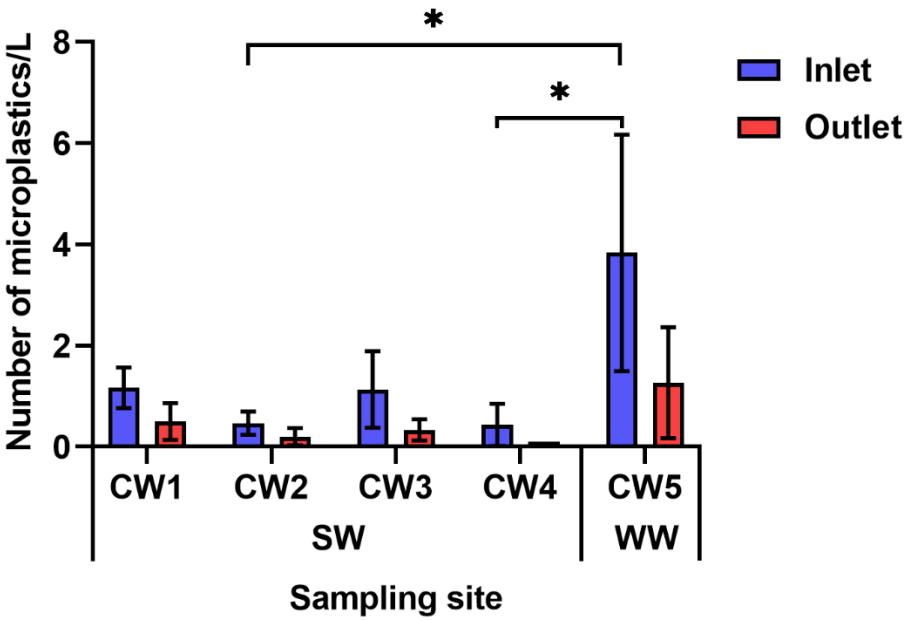
676 Yuan, W. K., Liu, X. N., Wang, W. F., Di, M. X., & Wang, J. (2019). Microplastic abundance,
677 distribution and composition in water, sediments, and wild fish from Poyang Lake,
678 China. *Ecotoxicology and Environmental Safety, 170*, 180-187.
679 doi:10.1016/j.ecoenv.2018.11.126
680 Zhang, L., Liu, J., Xie, Y., Zhong, S., Yang, B., Lu, D., & Zhong, Q. (2020a). Distribution of
681 microplastics in surface water and sediments of Qin river in Beibu Gulf, China. *Science*
682 *of the Total Environment, 708*. doi:10.1016/j.scitotenv.2019.135176
683 Zhang, X., Leng, Y., Liu, X., Huang, K., & Wang, J. (2019). Microplastics' pollution and risk
684 assessment in an urban river: A case study in the Yongjiang River, Nanning City, South
685 China. *Exposure and Health*. doi:10.1007/s12403-018-00296-3
686 Zhang, Y. L., Kang, S. C., Allen, S., Allen, D., Gao, T. G., & Sillanpaa, M. (2020b).
687 Atmospheric microplastics: A review on current status and perspectives. *Earth-Science*
688 *Reviews, 203*. doi:10.1016/j.earscirev.2020.103118
689 Zhou, X., Zhao, Y., Pang, G., Jia, X., Song, Y., Guo, A., Wang, A., Zhang, S., & Ji, M. (2022).
690 Microplastic abundance, characteristics and removal in large-scale multi-stage
691 constructed wetlands for effluent polishing in northern China. *Chemical Engineering*
692 *Journal, 430*. doi:10.1016/j.cej.2021.132752
693 Ziajahromi, S., Drapper, D., Hornbuckle, A., Rintoul, L., & Leusch, F. D. L. (2020).
694 Microplastic pollution in a stormwater floating treatment wetland: Detection of tyre
695 particles in sediment. *Science of the Total Environment, 713*.
696 doi:10.1016/j.scitotenv.2019.136356
697 Ziajahromi, S., Kumar, A., Neale, P. A., & Leusch, F. D. L. (2018). Environmentally relevant
698 concentrations of polyethylene microplastics negatively impact the survival, growth
699 and emergence of sediment-dwelling invertebrates. *Environmental Pollution, 236*, 425-
700 431. doi:10.1016/j.envpol.2018.01.094
701 Ziajahromi, S., Neale, P. A., & Leusch, F. D. L. (2016). Wastewater treatment plant effluent
702 as a source of microplastics: review of the fate, chemical interactions and potential risks
703 to aquatic organisms. *Water Science and Technology, 74*(10), 2253-2269.
704 doi:10.2166/wst.2016.414
705 Ziajahromi, S., Neale, P. A., Rintoul, L., & Leusch, F. D. L. (2017). Wastewater treatment
706 plants as a pathway for microplastics: Development of a new approach to sample
707 wastewater-based microplastics. *Water Research, 112*, 93-99.
708 doi:10.1016/j.watres.2017.01.042
709 Ziajahromi, S., Neale, P. A., Silveira, I. T., Chua, A., & Leusch, F. D. L. (2021). An audit of
710 microplastic abundance throughout three Australian wastewater treatment plants.
711 *Chemosphere, 263*. doi:10.1016/j.chemosphere.2020.128294

712



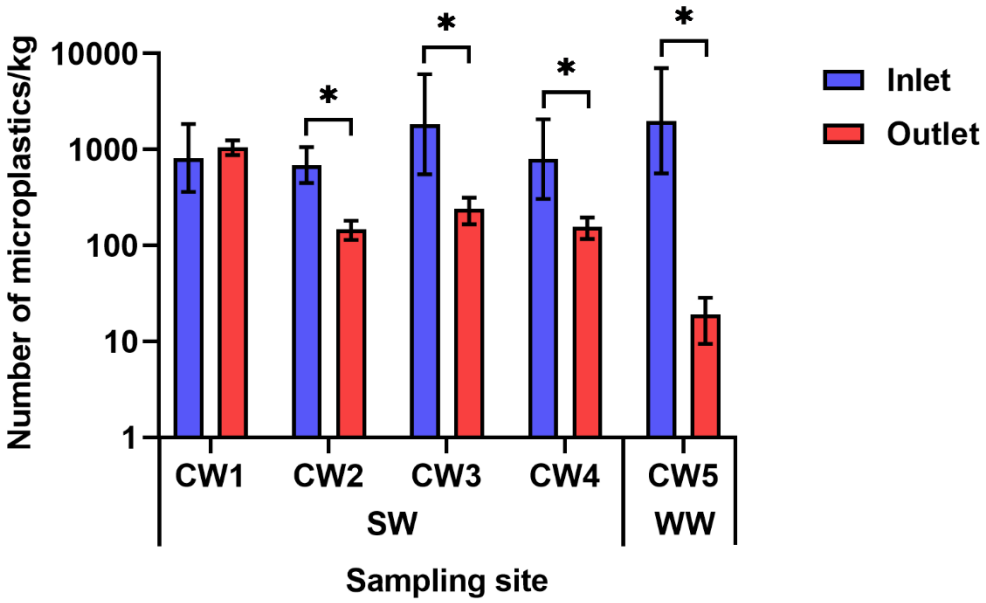
713

714 **Figure 1.** Location of sampling constructed wetlands (CWs).



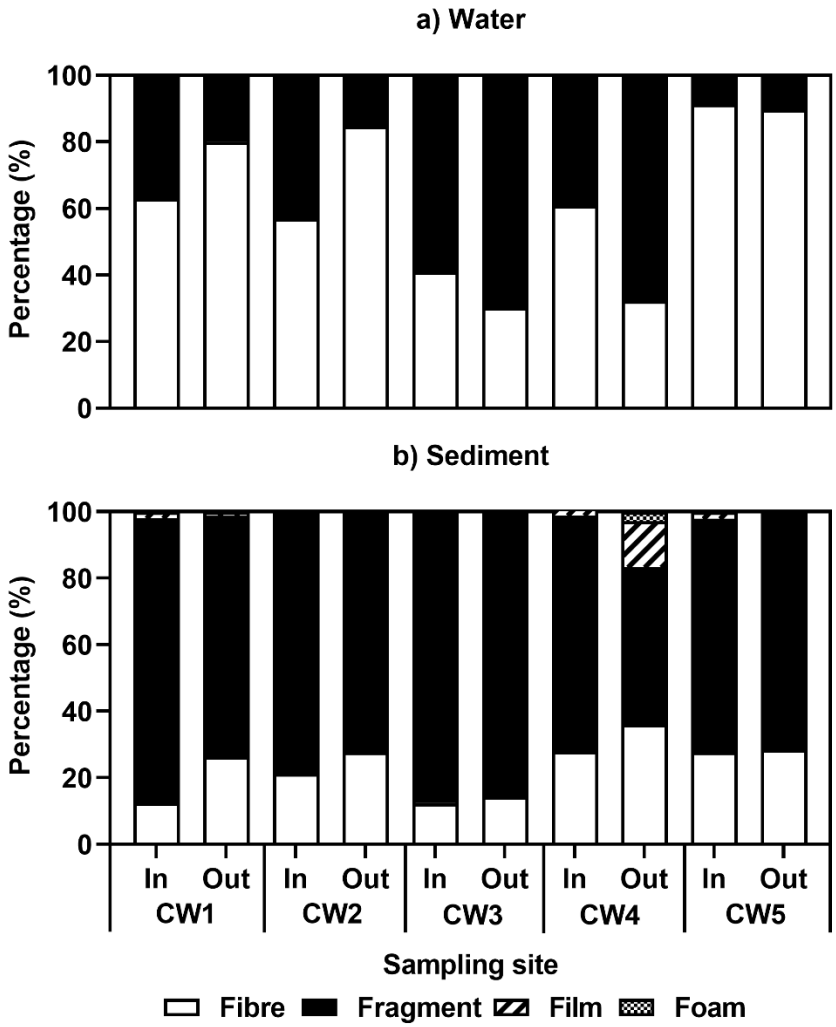
715

716 **Figure 2.** Number of microplastics per litre of water at the inlet and outlet of constructed
717 wetland (CW). Data are presented as the average of replicates (n = 3), with error bars indicating
718 standard deviation. SW: stormwater; WW: wastewater. * denotes significant differences.



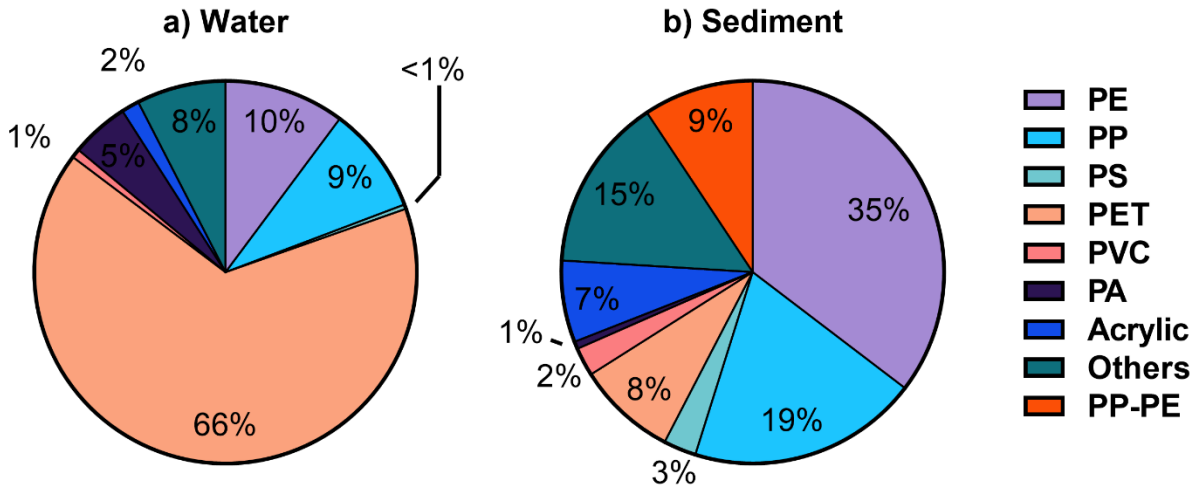
719

720 **Figure 3.** Number of microplastics per kg dry sediment at inlet and outlet of constructed
721 wetlands. Data are displayed on a log scale and presented as the average of replicates, with
722 error bars indicating standard deviation. * denotes significant differences.



723

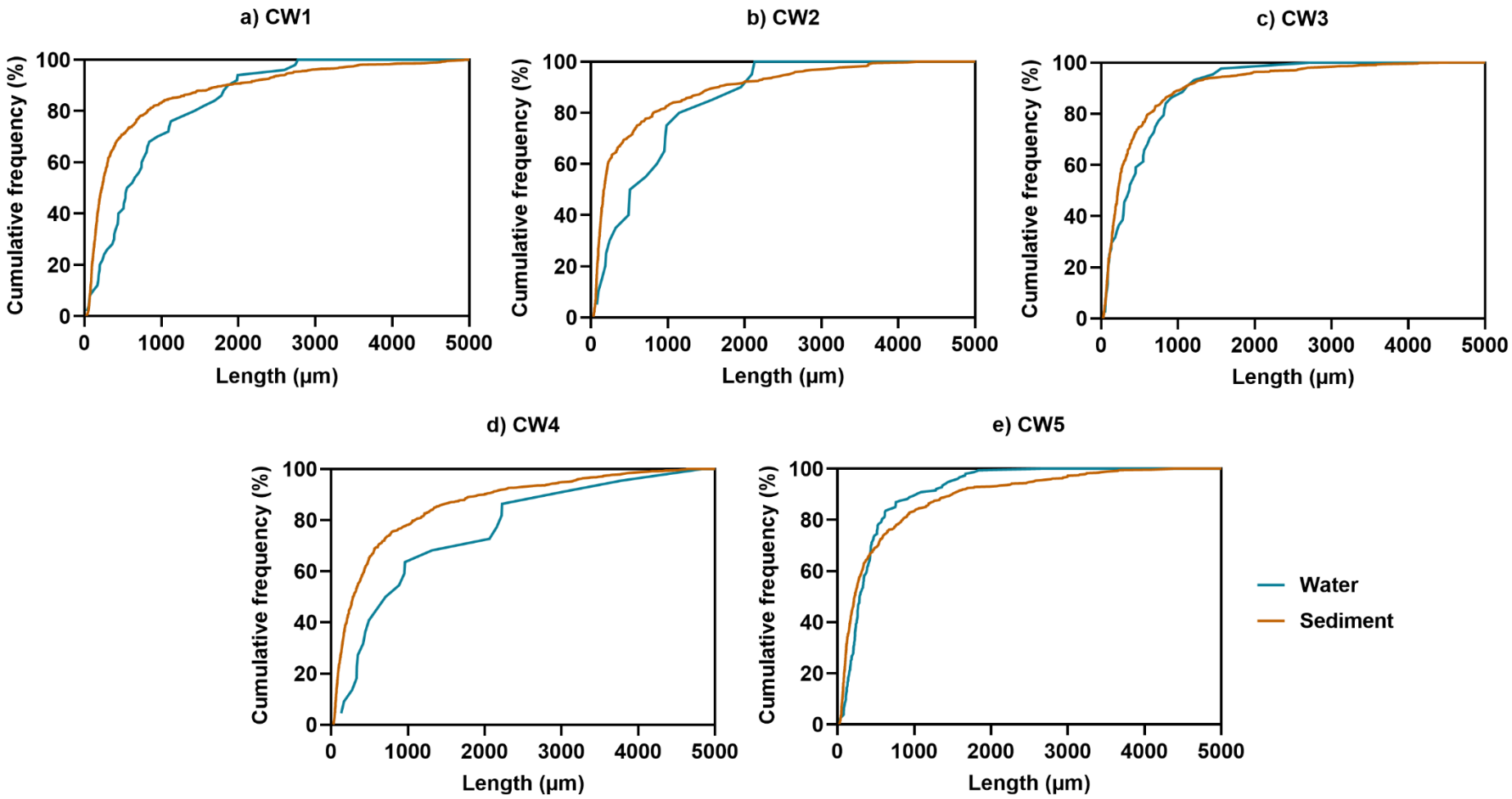
724 **Figure 4.** Percentage of different shapes of microplastics detected in the a) water and b)
725 sediment of the constructed wetlands (CW). Data presented as the average of replicates.



726

727 **Figure 5.** Percentage of different microplastic polymers detected in a) water and b) sediment

728 of the constructed wetlands. Data presented as the average of replicates.



729

730 **Figure 6.** The distribution of microplastic size in water and sediment at five constructed wetlands.