



# Abundance and variation of microplastics between seasons in a tropical estuary: The case of Can Gio estuary, Vietnam

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**Abstract:** Every year, millions of tons of plastic waste are dumped into the ocean from estuaries. The process of accumulating and converting plastic into microplastics (MPs) in this dynamic system has not received as much attention compared to the open-ocean region. Therefore, this study aimed at evaluating the seasonal variation in microplastic (MP) content at the Can Gio estuary, Vietnam, during the rainy and dry seasons of 2021 and 2022. Water, sediment, and biological samples were collected at four sites. MP contents ranged from  $0.00134 \pm 0.00043$  to  $0.00095 \pm 0.00014$  items/L in water surface samples, from  $4.22 \pm 0.46$  to  $2.44 \pm 0.46$  items/L in water column samples, and from  $200 \pm 13.68$  to  $90 \pm 13.68$  items/kg in sediment samples. There was an interactive effect of seasonal fluctuation and the complex flow regime on the MP content. MP bioaccumulation in *Saccostrea* and *Periophthalmodon schlosseri* was 1.5 – 1.8 and 0.2 – 0.52 items/individual, respectively. The main MP shapes were fibers and fragments. The composition mainly consisted of polypropylene (45.45%), polyethylene (18.18%), polystyrene (9.09%) and other plastics (27.28%). The source of MPs can be projected from the macro-plastic stream accumulated at the sampling sites. This study found the presence of MPs in both seasons of the year, and the accumulation of MPs in this dynamic region is closely correlated with hydrological properties of the estuary.

## Introduction

Estuaries are vital ecosystems that serve as transition zones between freshwater and saltwater, supporting a diverse range of biodiversity and providing essential ecosystem services (Levin et al. 2001). However, these sensitive environments are increasingly threatened by microplastic (MP) pollution (Horton and Barnes 2020). Estuaries act as sinks for microplastics (MPs) coming from rivers and coastal areas, making them hotspots for MP accumulation (Wang et al. 2022). MPs pose a significant threat to marine life, potentially leading to ingestion, entanglement, and toxicity (Gola et al. 2021). Understanding the distribution, abundance, and potential impacts of MPs in estuaries is crucial for developing effective mitigation strategies to protect these valuable ecosystems (Wang et al. 2022).

MPs can be released into the aquatic environment as primary pollutants produced by the cosmetic industry or transformed from macro-plastics through the combined effects of physico-chemical-biological changes. MPs are often covered by biofilm, which plays an important role in their continued degradation or alters the plastics' toxicity. The continuous decomposition of MPs in the aquatic environment results in changes to their

physical characteristics, such as color, surface morphology, size, crystallinity, and density (Tirkey and Upadhyay 2021). These modifications may alter their chemical and physical activity, as well as change their impact on the environment and life forms (Kye et al. 2023, Guo and Wang 2019a). Biofilm formation can significantly increase the accumulation of pollutants on plastic surfaces compared to virgin plastics, contributing to the accumulation of environmental pollutants in the aquatic environment (Bhagwat et al. 2021, Guo and Wang 2019a, Wang, Guo, and Xue 2021). A recent review of Zhang (Zhuang and Wang 2023) reported that aged MPs with attached biofilm could absorb more types and quantities of antibiotics, enhancing the biodegradation of some antibiotics in the aquatic environment. MPs with established biofilm increase the risk of bioaccumulation by marine animals (such as fish, birds, sea turtles, and crustaceans) because they mistake plastic fragments for food (Roman et al. 2021). Other ecological risks come from the release of additives used in the plastic manufacturing process (He et al. 2023). The release of additives during the MPs aging process is influenced by the physical – chemical conditions of the aquatic environment, such as pH, salinity, and temperature (Zha et al. 2022, Chen et al. 2019, Bakir et al. 2014).

MPs are carried by currents and tides until they accumulate in the water, sediment, or living organisms. In estuary regions, MP accumulation is influenced by strong turbulence and the interactions with sediment and biological aggregates (Wang et al. 2022). The hydrological regime in these dynamic regions differs between seasons, with lower tidal and water levels during the dry season, and higher levels during the rainy season. Salinity and salt intrusion also vary between seasons, creating different physical environments in the estuary (Vu Thi 2019). A study of Dalu et al. reported the variation of MPs between seasons in river sediment, but no clear difference was found in the water (Dalu et al. 2023).

Related to the sources of MPs in the marine environment, Asia is responsible for more than 80 % of plastic leakages into the oceans, with eight out of the top ten contributing countries being from this region, and Vietnam ranking fourth globally (World Bank Group 2021). A study conducted in August 2020 (before the COVID-19 pandemic) by Nguyen et al., found MPs concentrations in the Sai Gon river and Can Gio sea to be 2.08 and 0.6 pieces/m<sup>3</sup>, respectively (Nguyen et al. 2022). Another study conducted from February 2020 to March 2021 (Khuyen et al. 2022) reported a concentration of 10 items/L in the Sai Gon river water. MPs have become emergent transboundary pollutants. More frequent monitoring of their abundance, sources, transport, and bioaccumulation is needed. The objectives of this study are assessing the abundance and variation of MPs between dry season and rainy season and exploring the MPs distribution among different environmental compartments in the tropical estuary Can Gio.

## Material & Methods

### Study area

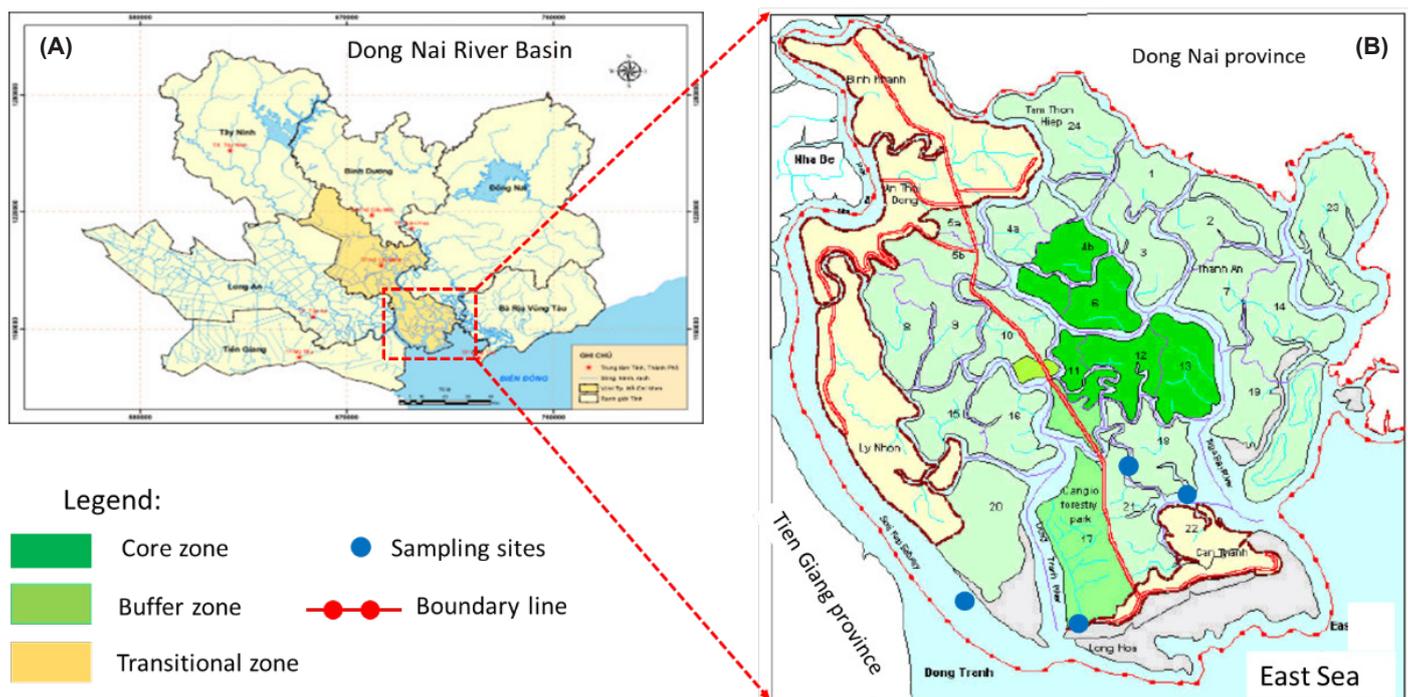
Can Gio is a coastal district region southeast of Ho Chi Minh City, a mega city with a population of more than 9 million (GSO 2021). Can Gio possesses the Can Gio Mangrove Biosphere

Reserve (CMBR), with a total area of 704.45 km<sup>2</sup> (Nam et al. 2014) (Figure 1). Intensive aquaculture farming (oysters, shrimp, etc.) is being developed near coastal towns (Cormier-Salem et al. 2017). The shape of Can Gio resembles a triangle, with two sides formed by the Soai Rap and Thi Vai rivers, and the third side formed by the Can Gio coastline (Figure 1B). Located downstream of the Dong Nai River Basin, Can Gio is a low-lying delta formed by alluvial deposition from big rivers, including the Soai Rap, Dong Tranh, Long Tau, Thi Vai (Tuan and Kuenzer 2012). This region is in a tropical and monsoonal climatic zone; therefore, the hydrological regime and salinity differ between the rainy season (from June to October) and the dry season (from November to May) (Vu Thi 2019).

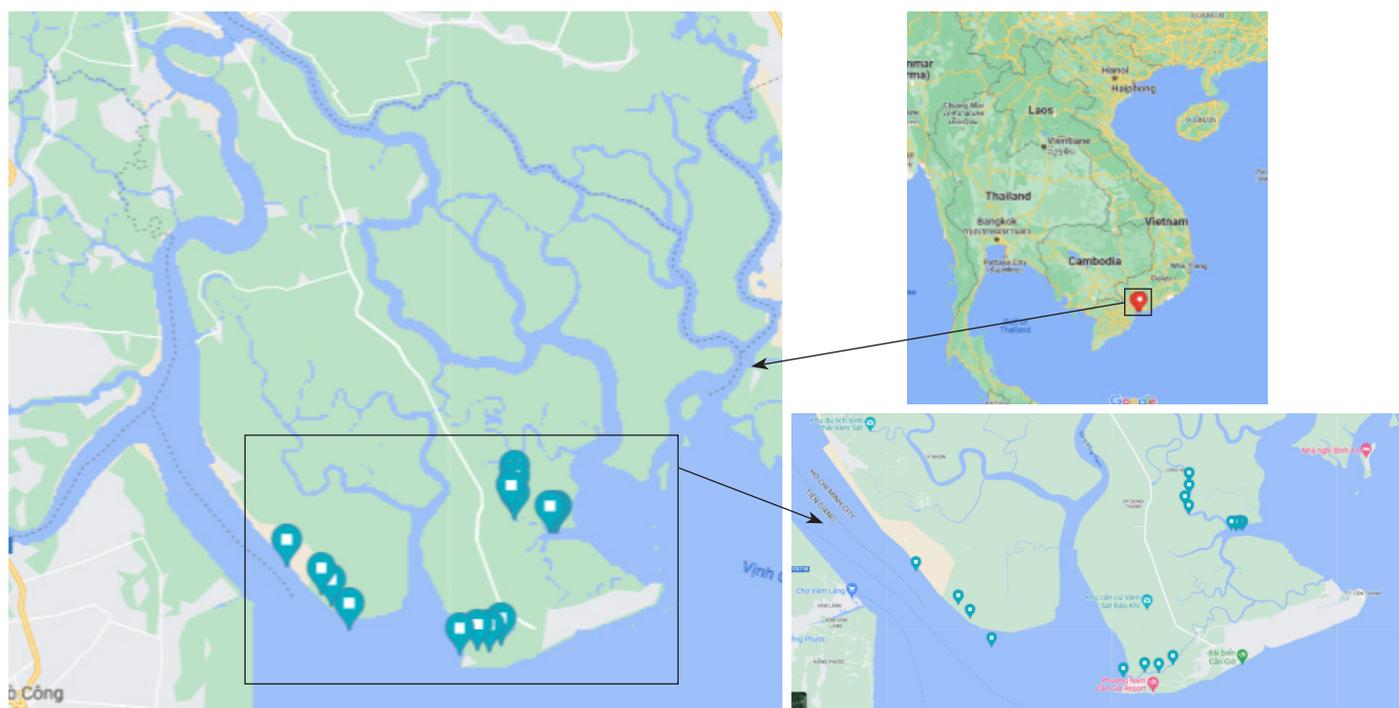
The risk of MP bioaccumulation in this biodiversity hotspot is of great concern. In this research, we studied the accumulation of MPs in local species with different feeding habits: the oyster (*Saccostrea*) and the mudskipper (*Periophthalmodon schlosseri*). Mudskipper is a natural gobiid fish that lives in the intertidal habitat of mudflats and mangrove ecosystems. It feeds actively in the mudflats during low tide. According to Bob-Manuel (2011), the feeding habits of mudskippers (*Periophthalmus koelreuteri*) change as they mature. During the juvenile stage, they are herbivorous, feeding mainly on aquatic macrophytes, diatoms, and algal filaments. As adults, their diet shifts to crustaceans, aquatic and terrestrial insects and polychaetes. Oysters are suspension feeders, capturing and ingesting food particles suspended in water, including phytoplankton, zooplankton, bacteria, and detritus (Pouvreau, Bodoy, and Buestel 2000). Oysters are raised by farmers in this estuary and are two important species in this ecosystem food web.

### Samples Collection

Sampling was conducted at four sites in the Can Gio estuary (Figure 2). Site information is given in Table 1. The river level at the four sampling sites was defined based on the flow



**Figure 1.** A. Location of Ho Chi Minh City within the Dong Nai River basin; B. Can Gio Estuary, Can Gio Mangrove forest zones, and sampling sites



**Figure 2.** Can Gio Estuary and the sampling sites in Can Gio, Ho Chi Minh City, Vietnam. Four sampling sites (SR, DH, DD, LH) were sampled in both seasons (Picture: earth.google.com)

rate measured at these locations from other studies (Vu Thi 2019, Nguyen Huu 2000). Sampling was done for two seasons after the COVID pandemic lockdown in Vietnam. The first sampling was conducted in the dry season (December 2021), which coincided with the Southwest and Northeast monsoons. The second sampling was done in the rainy season (May to June 2022), which coincided with the Northeast and Northwest monsoons. At each site, three continuous transects, each with 10 mins of continuous towing by ship, were sampled. Three types of samples, including water surface, water column, and bottom sediment, were collected in triplicates at each transect.

Water surface samples were collected using the net trawling method (Masura et al. 2015). Two Wisconsin samplers (Nitex SKU 40-A40, mesh size 63  $\mu\text{m}$ , mouth diameter 130 mm, ring diameter 180 mm, length from the two-panel cod-end 42 cm) were attached to the sides of the boat with brand-new ropes. The rope length was adjusted to set up the two plankton

nets outside the wake zone generated by the board to prevent interference from water turbulence. The inflow area of the nets was controlled by maintaining a stable immersion depth of 0.08 m. The boat's speed was maintained at about 1.8 m/s during the towing process using a flow meter (JDC Electronics Flowwatch Flowmeter). After towing, the contents of both nets were washed through two sieves, 5 mm and 0.022 mm, using distilled water. All particles retained on the 0.022 mm sieve were transferred to a glass bottle using distilled water and brought to the laboratory for MP analysis. The contents of both nets were combined into one sample for each sampling site.

Water column samples were collected at a depth of 5 m (Masura et al. 2015). Sampling locations for the water column were at the midpoint between mid-stream and the shore, to be at a lower hydrodynamic exchange than in the mid-flow. An Alpha Water Sampler (SKU 1120-C42, 2.2 L), tied with a rope marked at 5 m, was used. The sampler was dropped vertically until the

**Table 1.** Information of sampling sites for MPs at Can Gio Estuary

Site Number	Site code	Site name	Environment	Start attitude – End latitude	Start longitude – End longitude	Distance to the main river stream (m)	River level*	Surrounding anthropogenic activities
1	DD	Dong Dinh	Long Tau Estuary	10.4399381 – 10.4398916	106.9216654 – 106.9261239	1,720.0	2	Port, Marine transportation
2	LH	Long Hoa	Long Hoa River	10.4541077 – 10.4460696	106.9051044 – 106.9050002	5,997.8	3	Oyster farming
3	DH	Dong Hoa	Dong Tranh Estuary	10.383099 – 10.3879099	106.8794605 – 106.8987707	0	2	Aquaculture, Port, Households
4	SR	Soai Rap	Soai Rap Estuary	10.3948877 – 10.424253	106.827998 – 106.798452	0	1	Near industrial zones, Marine transportation

\* River level classified based on other studies measured at this region (Nguyen Huu, 2000; Vu Thi 2019), in which classified into scale of 1 to 3. River level 1 is the large estuary of the main stream, having strong flow. River level 2 is the river connecting to the main stream. River level 3 is river connecting to the river level 2.

marked depth was reached. The device filled with water at 5 m depth and was then pulled up. A composite sample of the water column with a total volume of 10 L was collected at each site. The collected water was filtered through two sieves of 5 mm and 0.022 mm. The fraction retained on the 0.022 mm sieve was transferred to a glass bottle for later analysis. Blanks were prepared with distilled water poured into glass bottles for later analysis.

The bottom sediment samples were taken at the same locations as the water column samples. Bottom sediment samples were collected by dropping a Grab Sampler (Petite Ponar 1728-G30) vertically from the side of the boat until the bucket touched the riverbed. The sampling bucket has a volume of 2.4 L and a sampling area of  $15 \times 15$  cm. The bucket automatically unlatched and retained the top layer of sediment inside. The bottom sludge sample contained in the bucket was completely transferred into a new clean zip bag made of low-density polyethylene. At each site, triplicated sediment samples, each with a volume of about 2L, were collected. All sampling tools and containers were washed thoroughly with distilled water three times before each sampling to prevent cross contamination. Field blanks were made for each sampling location.

Local biological samples, including oysters (*Saccostrea*) and mudskippers (*Periophthalmodon schlosseri*), were collected for MPs accumulation analysis. The composite bio-samples from different locations in Can Gio were collected by local farmers. Fifteen individual oysters and twenty-five individual mudskippers per season were collected. Five replicates, each containing three oysters or five mudskippers, were analyzed. The criteria for selecting both oysters and mudskippers from different locations in the study site were based on size and maturity. Mature oysters, approximately 20 cm in length, and mature mudskippers with a head size of about 4 cm were collected. The animal samples were prewashed at the site, transferred to clean zip bags, and placed in an ice bucket for transport back to the laboratory. All biological samples were stored in the freezer at  $-20$  °C until testing.

### **Analyzing environmental conditions of the sampling sites**

Environmental conditions at the sampling sites were recorded to characterize the sampling conditions. pH, salinity, total dissolved solids (TDS), dissolved oxygen and temperature were measured onsite by YSI Pro Plus Water Quality Instrument. Wind speed and flow velocity were measured using an FW450 Flow Watch-Ntech.

### **MPs separation**

MPs in all sample types were analyzed using the flotation method of the National Oceanic and Atmospheric Administration (NOAA) (Masura et al. 2015). The collected water samples were first filtered through a 5 mm sieve, and then through a 0.022 mm sieve. All particles retained on the 0.022 mm sieve were dried at 60 °C, then oxidized with a solutions of 0.5 M  $\text{FeSO}_4$  and 30 %  $\text{H}_2\text{O}_2$  to remove the attached organic matter. After oxidation, the suspension was filtered through 0.45  $\mu\text{m}$  filter paper (cellulose nitrate Satorius 11406-47-CAN) and then subjected to a flotation step using  $\text{ZnCl}_2$  solution (density of  $1.5 \text{ g.cm}^3$ ). The supernatant was separated and filtered through 0.45  $\mu\text{m}$  filter paper. The filter paper was stored in a tightly sealed petri dish for further analysis.

The sediment samples were dried at 60 °C until a constant mass was achieved, then sieved through a 5 mm sieve to remove all particles larger than 5 mm. The flotation and oxidation steps for the sediment samples followed similar protocol as for the water samples. MPs were collected onto 0.45  $\mu\text{m}$  filter paper. The  $\text{ZnCl}_2$  salt solution was reused by filtering it through 0.45  $\mu\text{m}$  filter paper.

Extraction of MPs from oysters was hindered by the presence of fats that float on top of the aqueous solution, which can interfere with the correct identification and counting of MPs in the sample. Therefore, oxidation and flotation steps were repeated several times if samples contained a lot of organic matter or fats. For the oyster samples, the gastrointestinal tract (GIT), including gills and soft tissue, was dissected and collected for MP extraction. For fish samples, the digestive tract and gills were separated for MP extraction. The twenty-five mudskippers were divided into five replicates (each replicate with 5 individuals). For the oysters, the fifteen individuals were divided into five replicates (each with 3 individuals). Oxidation and flotation steps for each replicate followed similar protocol as for the water and sediment samples.

### **MPs quantification**

The MPs on filter papers were examined using a stereomicroscope (LW Scientific, USA) with a zoom magnification range from 6.5X to 45X, following the method described by Ivar do Sul (Ivar do Sul and Costa 2014). Images of the MPs were captured using an iPhone 11 Pro Max. The number of MPs on each filter paper was counted manually, with the lower size limit for this method being 0.05 mm. MPs were classified into five groups based on their morphological characteristics: fiber, film, fragment, foam, and bead. The color and size of the MPs were measured by randomly selecting an equal number of grids on each sample filter for quantification. The color of the MPs was determined by visual examination through a light microscope, and the longest dimension of each microplastic particle was measured with a digital ruler to determine the size. To determine the composition of MPs, forceps were used to pick up MPs ranging in size from 2 to 5 mm for analysis with an ATR-FTIR (Bruker Vertex 70 instrument). The spectra of the MPs were then compared with libraries in SpectraBase (<https://spectrabase.com/>) and siMPle (<https://simple-plastics.eu/>) for plastic polymer identification. The FTIR spectra of the samples were matched with the reference spectra in the database, using the spectral range ( $3996 - 599 \text{ cm}^{-1}$ ) and produced a score between 0 and 1, indicating the goodness of fit.

### **Quality assurance and quality control**

The sampling procedure was carried out according to the latest quality assurance and quality control requirements (Adomat and Grischek 2021, Hermsen et al. 2018, Koelmans et al. 2019). During the sampling and laboratory procedures, all solutions, including  $\text{ZnCl}_2$ , distilled water,  $\text{H}_2\text{O}_2$ , and  $\text{FeSO}_4$  were filtered through a 0.45  $\mu\text{m}$  Cellulose Nitrate filter (Satorius 11406-47-ACN) before use. Every laboratory apparatus was thoroughly cleaned with distilled water that had been filtered three times and then left to dry on an aluminum-covered test bench. Three blanks were used for each medium in each experiment. The laboratory blanks were filled with filtered distilled water and

processed using the same method as the sample treatment (Zhao et al. 2021). The final abundances were adjusted based on the mean value of microplastic pieces found in the respective blanks.

### Data Analysis

The abundance of MPs in water surface samples was determined by dividing the number of plastic particles detected by the total volume of filtered water collected by the two nets (items/m<sup>3</sup>). The volume of filtered water was calculated based on following equation:

$$\text{Total volume of filtered water (m}^3\text{)} = \text{tow distance (m)} \times \text{area of opening frame (m}^2\text{)}$$

In which:

– tow distance (m) = speed of boat (m/s) × duration (mins)

– area of opening frame for each net was 0.0096 m<sup>2</sup>

Total volume of filtered water at each site ranged from 20.7 to 34.5 m<sup>3</sup>. MPs in water column samples were recorded in item/L. The MPs in sediment samples were quantified in terms of items/kg dry weight (items/kg). The abundance of MPs in mudskippers and oysters was reported in terms of items per individual, and items per kg of wet body mass. Statistical analysis was done using JMP Pro 14. A full factorial analysis was conducted to estimate the main effects and interactions. Tukey's HSD test was carried out to compare means with  $\alpha = 0.05$ , with significant differences at  $p < 0.05$ .

## Result and discussion

### Environmental conditions at the study sites

Environmental conditions of the studied area are summarized in Table 2. There was a difference in salinity between sampling

points, with the lowest salinity at SR (13.3 – 15.0 ‰). The three other points had salinity in the range of 22.4 to 23.7 (‰). The pH ranged from 8.48 in the dry season to 7.69 in the rainy season. Temperature was 28.03 in the dry season and 28.20 °C in the rainy season. There was no significant difference in both temperature and pH between sites and between seasons. However, wind direction varied between sites and between seasons.

The stability or extent of aggregation of MPs in aqueous solutions is strongly affected by pH and salinity. According to the study of Sharma (Sharma et al. 2021), at pH levels from 5 to 9, the particle size of PE remained constant (from 284.92 to 310.29 nm) in a 5.84 % NaCl solution due to strong electrostatic repulsive forces. However, with pH levels from 6.3 to 6.8, four types of PS nanoparticles were found to be stable in the aqueous solution with a low NaCl concentration (< 5 ‰), but they started to aggregate irreversibly when the NaCl concentration raised from 10 ‰ to 35 ‰. In the Can Gio Estuary, pH varied in the range of 7.6 to 8.4, and salinity varied from 13.9 to 23.9 ‰. These conditions could allow the aggregation of MPs in the aqueous environment. However, the contribution of each factor cannot be concluded from this study due to the potential combined effect between factors or the potential interference of different types of MPs present.

### Identification and characteristics of MPs

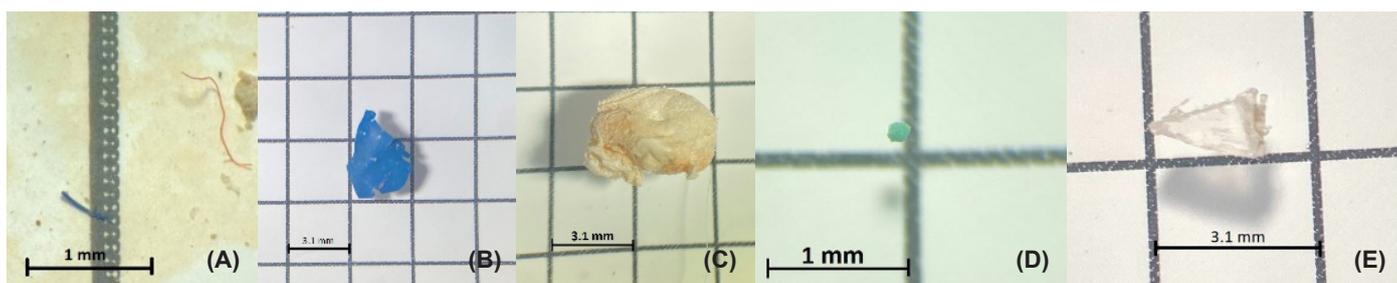
#### MPs shape, color and size

The total number of water and sediment samples in both seasons was 72. MPs in each sample were collected on one filter. The total number of MPs pieces in all 72 samples was 1894. Color and size were recorded by randomly selecting an equal number of grids on every membrane filter. The total number of MPs randomly selected from all 72 samples was 244 MP pieces. MPs in blank samples averaged  $0.27 \pm 0.13$  pieces

**Table 2.** Environmental conditions at the four studied sites in Can Gio Estuary. Each value is the average of two seasons. Each sampling site was measured at three transects without replication.

Site	Transect	Temperature (°C) (mean ± standard error)	Salinity (‰) (mean ± standard error)	pH (mean ± standard error)	Wind direction* (Dry season/rainy season)	Wind speed (m/s) (mean ± standard error)
DD	1	28.05±0.36	22.60±2.21	7.43±0.77	EW/EN	0.95±0.08
	2	28.50±0.36	23.13±2.21	7.69±0.77	EW/EN	1.04±0.08
	3	28.60±0.36	23.18±2.21	7.68±0.77	EW/EN	1.06±0.08
DH	1	27.85±0.36	23.73±2.21	7.67±0.77	W/-	1.46±0.11
	2	27.90±0.36	23.69±2.21	7.71±0.77	W/-	1.60±0.11
	3	27.40±0.50	24.16±2.21	9.19±0.77	W/-	1.77±0.11
LH	1	28.25±0.36	22.44±2.21	7.66±0.77	SE/EN	0.90±0.08
	2	28.50±0.36	22.93±2.21	7.65±0.77	SE/EN	0.86±0.08
	3	28.40±0.36	22.96±2.21	9.15±0.77	SE/EN	0.82±0.08
SR	1	27.40±0.36	15.03±2.21	9.11±0.77	S/-	0.83±0.12
	2	28.10±0.36	13.48±2.21	8.52±0.77	S/-	0.83±0.12
	3	28.15±0.36	13.35±2.21	7.60±0.77	S/-	0.83±0.12

\* EW: East West; W: West, SE: South East; S: South



**Figure 3.** Five types of MPs found in the waters and sediment from the Can Gio Estuary. A: Fiber. B: Fragment. C: Foam. D: Bead, E: Film.

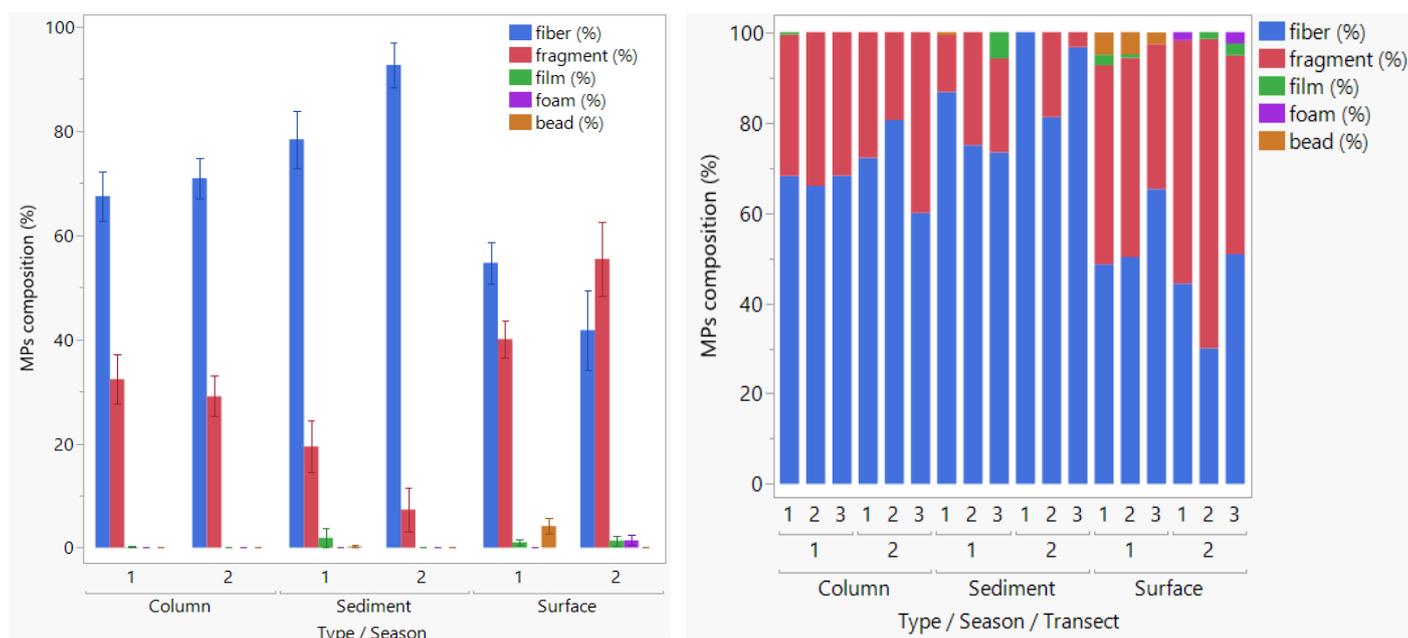
(mean  $\pm$  standard error). MP shapes were classified into five groups: fiber, fragment, film, foam, and bead (Figure 3). The color of each MP piece was categorized into white, yellow, blue, green, red, and black. Particle sizes were classified into four ranges: smaller than 0.3 mm, 0.3 - 0.5 mm, 0.5 - 1 mm, and 1 - 5 mm. The size ranges reported in this study, with some modifications, applied the classification of Crawford et al., who defined the size range of  $< 5\text{mm} - 1\text{mm}$  as MP, and  $< 1\text{mm} - 1\ \mu\text{m}$  as mini-MP (Crawford and Quinn 2017). MPs' sizes were determined based on the longest dimension of the particles measured with a digital ruler (JR Screen ruler) on the computer. The smallest size observed in all samples was 0.1 mm.

Fibers were the most abundant type of MPs in the water column and sediment samples in both seasons. In the water surface samples, fibers were the most dominant component in the dry season, while fragments were more dominant than fibers in the rainy season (Figure 4). Fragments were the second most dominant shape of MPs in all samples except the water surface samples during rainy season. Films occurred in only one transect of sediment samples in the dry season but were found in water surface samples in both seasons. Foams were found in water surface samples in the rainy season only. Beads were only found in water surface samples in the dry season. The variation of MPs shapes between sample types

suggests that MPs' density and river flow rate influence their distribution (Borges-Ramírez et al. 2020).

The dominance of fiber MPs has also been found in several other studies across various marine areas, such as in marine fish in Thailand (Klangnurak and Chunnuyom 2020), sediment in coastal waters at Jakarta Bay, Indonesia (Takarina et al. 2022), surface water and sediment in the Yangtze Estuary, China (Li et al. 2020), surface and sediment in China's inland water systems (Fan et al. 2022), and surface water of the lower Yellow River near the estuary (Han et al. 2020). The abundance of fragments as the second most common type of MPs has also been reported in some studies (Dodson et al. 2020, Klangnurak and Chunnuyom 2020, Fan et al. 2022, Han et al. 2020). However, the less dominant shapes of MPs varied in other studies; for example, films were found to be the second most dominant type of MPs in one study (Takarina et al. 2022).

MPs were measured for their color and size by randomly selecting 244 pieces from a total of 1894 pieces on the filters. The color of MPs was determined by visual examination using a light microscope. Several colors were observed in all sample types, including blue, green, red, black, and white. However, white MPs were only found in the water column and water surfaces samples and were absent from sediment samples. Blue was the most dominant color in the



**Figure 4.** MPs composition in all samples. (A) MPs compositions of three types of locations in two seasons. Each error bar is constructed using 1 standard error from the mean. (B) MP composition of each sample is classified into sample types (Column, Sediment and Water surfaces) at the two seasons (1: Dry season, 2: Rainy season) at each transect (1, 2, 3).

**Table 3.** The proportion of MPs' colors in different sample types

MPs' color	Proportion of MPs' color in each sample type		
	MPs in water column (% in total 244 measurements)	MPs in water surface (% in total 244 measurements)	MPs in sediment (% in total 244 measurements)
White	1.7%	4.0%	0.0%
Black	1.7%	1.6%	8.3%
Red	29.3%	30.2%	48.3%
Green	6.9%	18.3%	8.3%
Blue	60.3%	46.0%	35.0%

**Table 4.** The proportion of MPs' size in different sample types.

Size range	Proportion of MPs' size in each sample type		
	MPs in water column (% in total 244 measurements)	MPs in water surface (% in total 244 measurements)	MPs in sediment (% in total 244 measurements)
<0.3 mm	43.1%	28.6%	21.7%
0.3-0.5 mm	24.1%	12.7%	15.0%
0.5-1 mm	8.6%	24.6%	35.0%
1-5 mm	24.1%	34.1%	28.3%

water column and water surface samples, followed by red and then green. Black and white accounted for the smallest percentage in all samples. In sediment samples, red was the most dominant color, followed by blue, with green and black being equally represented (Table 3).

The color of MPs varies significantly among studies (Montoto-Martinez, Hernandez-Brito, and Gelado-Caballero 2020). For instance, in the Yangtze Estuary, China, white MPs were found in surface water, and transparent MPs were found in sediment (Li et al. 2020). The color of MPs is influenced not only by the original color of the material but also by the aging process of plastics during MP formation (Zhao et al. 2022). The predominance of blue MPs may be linked to differences in plastic aging between colors (Zhao et al. 2022, Zha et al. 2022). Blue plastics do not effectively absorb UV light, which means they transmit more light energy compared to red or yellow plastics. As a result, blue plastics age faster in the sun, leading to a higher proportion of blue microplastics in the environment. The color of MPs can also indicate the state of plastic aging or the exposure time of plastic particles in the environment. During the extended exposure, the color of plastics first gradually changes to light colors (decolorization) and then further changes from white to yellow or amber due to weathering (Zha et al. 2022, Zhao et al. 2022). The very low proportion of white color (from 0 to 4%) and the absence of yellow color MPs in this study suggest a relatively low aging level of plastics in this estuary. This could also indicate that the MPs found in this area potentially originate from surrounding water bodies.

MP size was measured by their maximum dimensions and classified into four ranges: 1–5 mm, 0.5–1 mm, 0.3–0.5 mm, and < 0.3 mm. The size distribution of MPs differed among the three location types. Small MPs particles (< 0.3 mm and 0.3 to 0.5 mm), were the most dominant in the water surface samples. Larger MPs sizes were more commonly found in the water

column and sediment compared to the water surface (Table 4). The distribution of colors and sizes between sample types suggests potential circulation and accumulation of MPs among the water surface, water column, and sediment, influenced by MPs' properties (size and density) and river flow conditions (flow rate and mixing status) (Borges-Ramírez et al. 2020). This study found that the dominant size of MPs varied between sample types: the smallest particles were distributed on the water surface, while larger particles were more prevalent in the lower layers (water column and sediment). Similar vertical size distribution patterns of MPs was reported by Fan (Fan et al. 2022). However, there is variation in the literature; for instance, smaller particles were found to be more dominant in sediment than in water surface samples in the Yangtze Estuary, China (Li et al. 2020). The size and color of MPs are influenced by the sources of primary MPs and the aging process of macro plastics, which leads to the formation of MPs in water (Zha et al. 2022). These factors are locally specific, contributing to variations observed between studies in different water bodies.

Attenuated Total Reflectance Fourier-Transform Infrared

**Table 5.** MPs present in the samples analyzed by ATR-FTIR. Matching score was obtained by comparing with the library in siMPLe\_ATR\_Version1.0.2 and spectra.com.

Plastic name	MP abundance (% in total 50 measurements)	Matching score
Polypropylene (PP)	45.45	0.65 – 0.69
Polyethylene_low_density (PE)	18.18	0.70 – 0.75
Polystyrene (PS)	9.09	0.62 – 0.80
Other plastics	27.28	

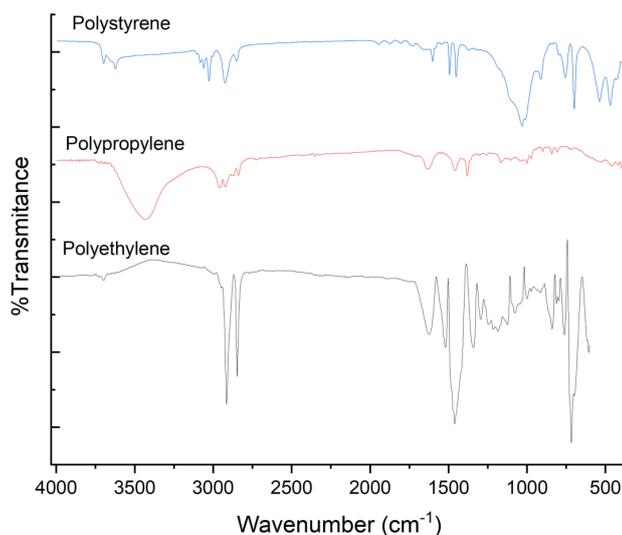


Figure 5. FT-IR spectra of MPs (size range of 2 – 5mm) found in all samples

Spectroscopy (ATR - FTIR) was used to determine the polymer types of MPs present in the samples. This method can detect MPs larger than 2 mm; MPs smaller than 2 mm could not be measured due to difficulties in sample preparation. The ATR-FTIR spectra identified major types of MPs, including polypropylene (PP), polyethylene (PE), polystyrene (PS), and other plastics (Figure 5). PP accounted for the largest proportion (45.45%), followed by PE (18.18%) and PS (9.09%) (Table 5). To further investigate the potential sources of MPs in this estuary, macro-plastic data was also recorded (detailed data not shown in this paper). The potential sources of the major MPs can be traced to the macro-plastic items found at the sites (Figure 6). Dominant macro-plastic types included fishing nets, ropes, foam boxes, plastic cans used for oyster farming, plastic boxes, and plastic bags. Broken pieces of these macro-plastics were also found and subsequently

contributed to the MPs stream. Figure 6 illustrates the potential sources of different types of MPs. As reported in other studies, the primary components of fishing nets and ropes are large-diameter PE, polyamide (PA) (nylon), and PP monofilaments (Guo and Wang 2019b). Over time, physical effects cause blue plastic cans to break down into smaller blue MPs fragments, while fishing nets break up into MP fibers.

**Variation of MPs within sample types and between seasons**

*MPs in the water surface*

The changes in MP content between dry and rainy seasons in the water surface are shown in Figure 7. The average MP concentrations were  $1.34 \pm 0.14$  in the dry season and  $0.95 \pm 0.14$  items/m<sup>3</sup> in the rainy season. The difference between seasons was most pronounced at LH River ( $p = 0.0287$ ),

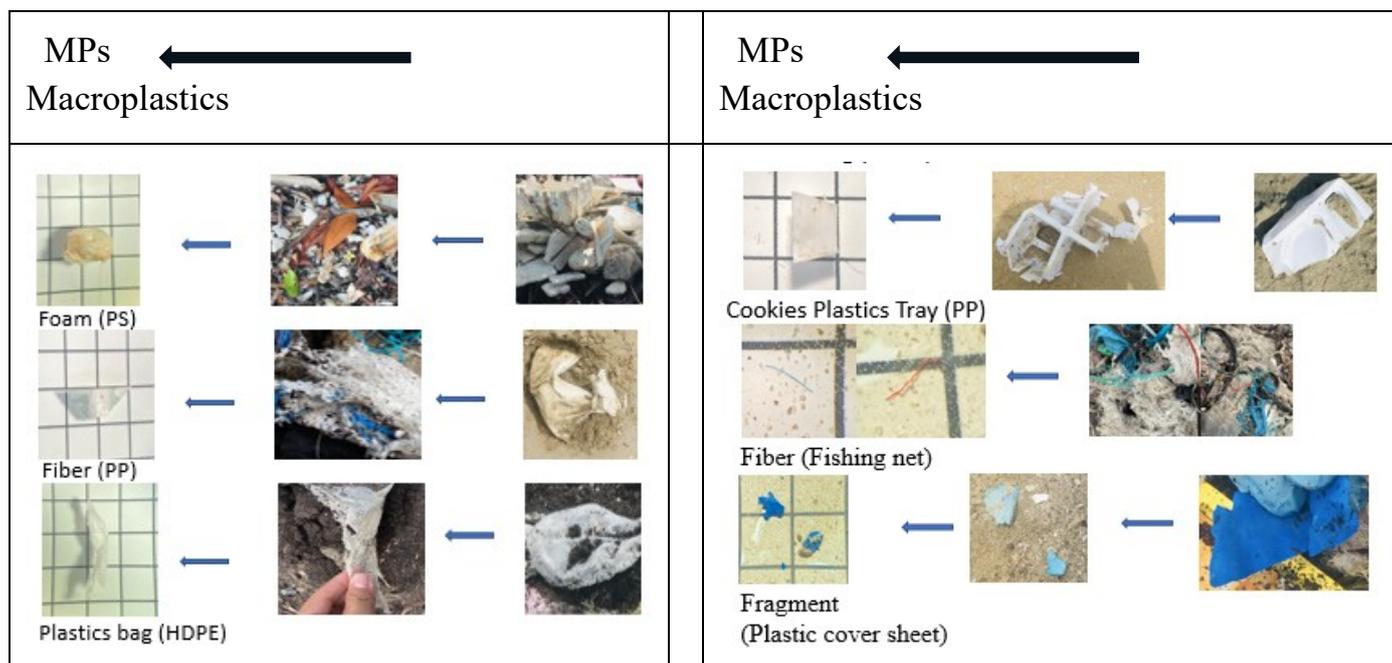
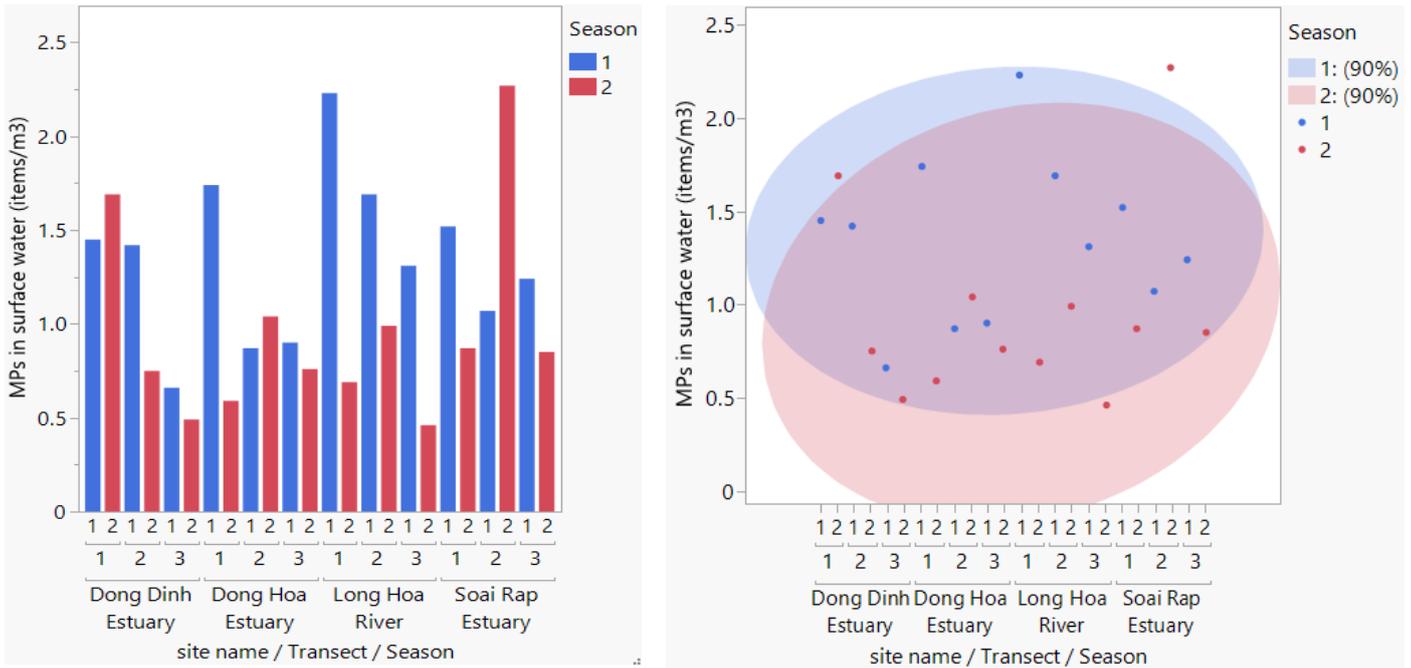


Figure 6. Predicted formation pathway of MPs from macro-plastics stream

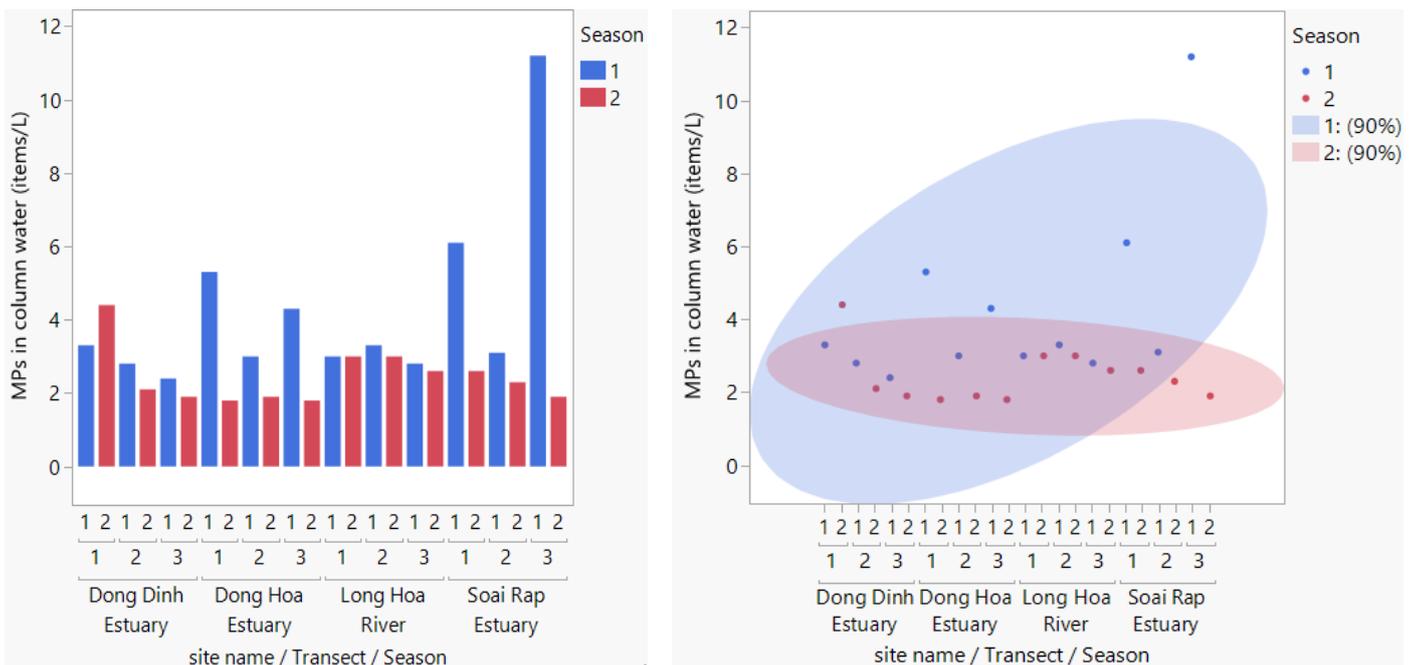


**Figure 7.** The distribution of MPs content in water surface during the dry season (Code 1) and wet season (Code 2). Picture on the right show density ellipsoid computed from the bivariate normal distribution with 90% coverage of the data point in each season, presenting the correlation of total number of MPs between seasons.

a river branch flowing into the mangrove area. At the other river estuary sites (Dong Dinh (DD), Dong Hoa (DH) and Soai Rap (SR)), no significant differences were detected between seasons. This data set shows that the influence of season on MP abundance in water surface samples was more pronounced in the river branch farther from the mainstream compared to the large rivers. In the dry season, MP content was higher in the river branch. Among the four sampling sites, LH, located in the river branch farthest from the mainstream, showed a more

visible seasonal impact than the other sites closer to or at the main stream.

Table 6 compares the MP abundance from different studies and the sampling methods they used. For MPs in surface water, several studies used plankton nets of varying pore sizes to trawl MPs. The average MP abundance in the surface water of Can Gio estuary for both seasons in this study was  $1.15 \pm 9.57$  items/m<sup>3</sup> (equivalent to  $14.37 \pm 119.62$  items/m<sup>2</sup>). This value is one to two orders of magnitude lower than the levels detected



**Figure 8.** The distribution of MPs content in the water column during the dry (Code 1) and rainy (Code 2) seasons. Picture on the right show density ellipsoid computed from the bivariate normal distribution with 90% coverage of the data point in each season, presenting the correlation of total number of MPs in water column between seasons.

**Table 6.** Comparison of MPs pollution at different river habitats in Asia.

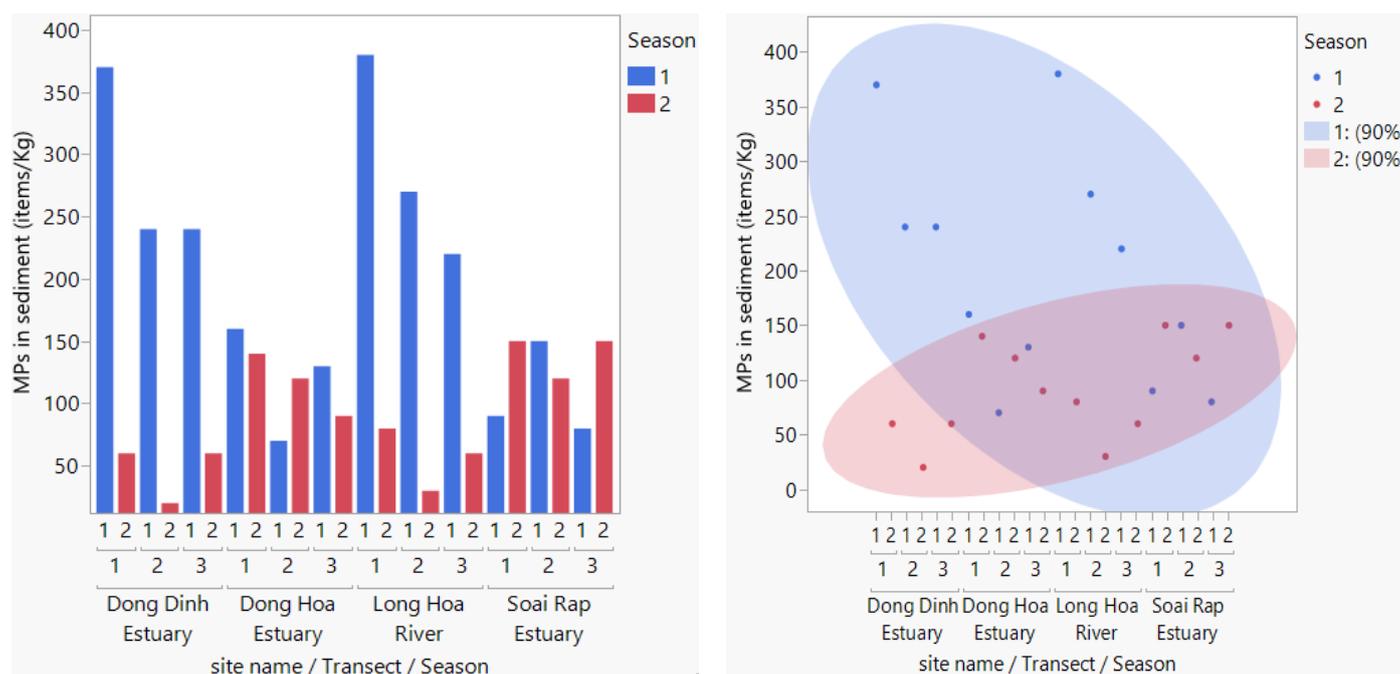
Study area	Habitat	Collection method	MPs in water (single values, range or mean $\pm$ standard deviation)	MPs in sediment (single values, range or mean $\pm$ standard deviation)	MPs in organisms (range or mean $\pm$ standard deviation)	References
Saigon River, Vietnam	Urban river	Grab 300 ml, Net 300 $\mu$ m	10–223 items/m <sup>3</sup>	/	/	(Lahens et al., 2018)
Ba Lat estuary, Viet Nam	Mangrove	Grab	/	646.2 $\pm$ 348.4 particles/kg	/	(Nguyen Trung, 2021)
Sai Gon, Dong Nai river, Viet Nam	Urban river	Grab, Net	228.120 items/L	38–498 items/kg	/	(Huynh et al., 2021)
Yangtze River, China	Urban river	Net. 300 $\mu$ m	0.49 items/m <sup>2</sup>	/	/	(Xiong et al., 2019)
Pearl River, China	Urban river	Net. 300 $\mu$ m	0.57 $\pm$ 0.71 items/L	685 $\pm$ 342 items/kg	/	(Fan et al., 2019)
Xiangxi River, China	Urban river	Net. 112 $\mu$ m	0.06–34.2 items/m <sup>2</sup>	80–864 items/m <sup>2</sup>	0–1.5 items/fish digestion tract	(Zhang et al., 2017)
Tapi-Phumduang River, Thailand	Rural river	Grab 5 L. 5 $\mu$ m	1.77 $\pm$ 0.28 items/L	55–160 items/kg	0–13 items/mussel 10–1.85 items/g mussel tissue	(Chinfak et al., 2021)
Ciwalengke River, Indonesia	Rural river	Grab 1 L. 1.2 $\mu$ m	5.85 $\pm$ 3.28 items/L	30.3 $\pm$ 15.9 items/kg	/	(Alam et al., 2019)
Dafeng River, China	Rural river	Net. 300 $\mu$ m	6 $\times$ 10 <sup>-4</sup> $\pm$ 5 $\times$ 10 <sup>-4</sup> items/L	14.1 $\pm$ 12.7 items/kg	2.2 $\times$ 10 <sup>3</sup> $\pm$ 1.6 $\times$ 10 <sup>3</sup> items/kg digestive tract tissue	(Liu et al., 2021)
Wei river, China		Grab 5L	3.67-10.7 items/L	/	/	(Ding et al., 2019)
Tuticorin coast of Gulf of Mannar, India	Sea	Grab 1L	10-30 items/L	/	/	(Sathish et al., 2020)
Port Dickson and estuary of Lukut, Malaysia	Coastal, estuarine	Net	2.10 to 6.80 items/L	/	/	(Zainuddin et al., 2022)
Jagir Estuary, Surabaya City, Indonesia	Estuary	Grab	/	414 particles/kg	/	(Firdaus et al., 2020)
Estuary in Can Gio, Viet Nam	Mangrove, Estuary	Net 63 $\mu$ m, Grab 10L, Van Veen grab	1.15 $\pm$ 9.57 pieces/m <sup>3</sup> , 3.33 $\pm$ 9.57 items/L	145.00 $\pm$ 9.57 items/kg	8.3 $\pm$ 1.6 items/kg (oyster) 1.6 $\pm$ 0.3 items / oyster digestive tract tissue 4.2 $\pm$ 4.4 items/kg (fish) 0.36 $\pm$ 0.37 items/fish digestive tract	This study

in the urban rivers upstream of this estuary (Table 6), including the Saigon River (10–223 items/m<sup>3</sup>) (Lahens et al. 2018), and the Dong Nai River (228.120 items/L) (Huynh et al. 2021) in Viet Nam. The Can Gio estuary receives discharges from the upstream Sai Gon River, Dong Nai River and Soai Rap River. These three large rivers with high flow rates facilitate MP transport and dilution in the estuary region. Studies in tropical coastal and estuarine zones in Malaysia found MPs at concentrations of 2.1 to 6.8 items/L (Zainuddin et al. 2022), which is higher compared to other Asian urbanized rivers, such as the Yangtze River in China 0.49 items/m<sup>2</sup> (Xiong et al. 2019) and the Xiangxi River in China 0.06–34.2 items/m<sup>2</sup> (Zhang et al. 2017). Nevertheless, all studies highlight the risk

of MP pollution in the surface water of various rivers, which can subsequently be transported to estuary and the ocean.

#### *MPs in the water column*

Figure 8 shows the difference in MP content in the water column between the dry and rainy seasons. Average MP concentrations in the water column were 4.22  $\pm$  0.46 items/L during the dry season and 2.44  $\pm$  0.46 items/L during the rainy season. MPs were significantly more abundant in the dry season compared to the rainy season ( $p = 0.0394$ ). The most pronounced difference was found in the large river estuaries (DH and SR), while no significant seasonal difference was found at LH river and DD Estuary (post-hoc analysis with  $\alpha$



**Figure 9.** The distribution of MP content in river sediment during the dry (Code 1) and rainy (Code 2) seasons. Picture on the right show density ellipsoid computed from the bivariate normal distribution with 90% coverage of the data point in each season, presenting the correlation of total number of MPs in sediment between seasons.

= 0.05). These results indicate that MPs in the water column of large rivers with high flow rates exhibit greater seasonal variability compared to river branches or tributaries with lower flow rates. This result contrasts with the results for the water surface. There was no significant interaction between season and river level affecting the abundance of MPs in the water column in the estuary region (full factorial analysis,  $p = 0.0779$ ).

The average microplastic abundance in the water column at Can Gio Estuary was  $3.3 \pm 9.75$  items/L, which is comparable to the MP concentrations observed in other urban rivers. For example, the Ciwalengke River in Indonesia had  $5.58 \pm 3.28$  items/L (Alam et al. 2019), the Wei river had 3.67-10.7 items/L (Ding et al. 2019), Tuticorin coast of the Gulf of Mannar in India had 10-30 items/L (Sathish, Jeyasanta, and Patterson 2020), and the Tapi-Phumduang River in Thailand had  $1.77 \pm 0.28$  items/L (Chinfak et al. 2021). These studies employed similar sampling methods, which facilitates comparison between different areas.

#### MPs in river sediment

Figure 9 compares MP abundance in river sediments between the dry and rainy seasons. The average concentration of MPs in the dry season was  $200.00 \pm 13.68$  items/kg, which was significantly higher than in rainy seasons ( $90.00 \pm 13.68$  items/kg) ( $p < 0.0001$ ). The seasonal influence on MP abundance in river sediments was most significant at LH and DD. LH is thae river branch with a lower flow rate than the main rivers and is located about 6,000 m from the mainstream. DD, also a tributary, is approximately 1,720 m from the mainstream and has a lower flow rate compared to the large estuaries SR and DH. The flow at the bottom in these areas is about 10-15% slower than at the surface, and turbulence level in the large estuaries (such as SR or DH) is higher than in the tributaries, particularly during the rainy season (Nguyen Huu 2000, Vu

Thi 2019). As a result, sedimentation in the large estuaries (SR and DH) may be lower than in the tributaries (DD and LH). MPs are thus likely to accumulate more in sediments in tributaries (DD and LH) during the dry season compared to the rainy season. There was a significant interaction between season and river level on MP abundance in the sediment of the estuary region (full factorial analysis,  $p = 0.00001$ ). The MP concentration in sediment at Can Gio Estuary, ( $145 \pm 9.57$  items/kg) is lower than in the Pearl River, China ( $685 \pm 342$  items/kg) (Fan et al. 2019) and the Xiangxi River, China (80–864 items/m<sup>2</sup>) (Zhang et al. 2017). It is comparable to the Tapi-Phumduang River, Thailand (55–160 items/kg) (Chinfak et al. 2021), but higher than in the Ciwalengke River, Indonesia ( $30.3 \pm 15.9$  items/kg) (Alam et al. 2019), and the Dafeng River - China ( $14.1 \pm 12.7$  items/kg) (Liu et al. 2021).

Sedimentation is also highly dependent on flow rate. The SR estuary is predominantly influenced by tidal currents, which account for more than 80% of the total flow and continuously change with tidal phases. The total flow rate here is usually greater than 60 cm/s, which exceeds the accretion speed of fine alluvium from upstream rivers, making it difficult for sediment to settle on the channel bottom. Additionally, the flow rate at the surface layer is usually 10-15% higher than at the bottom layer. In contrast, the flow in the Soai Rap estuary exhibits a complex spatial structure and varies with depth. At the surface layer, flow changes according to weather conditions, with wind being a significant factor affecting the water flow. From the subsurface to the bottom layer, the flow field structure changes with weather conditions (Nguyen Huu 2000). Among the four sampling sites, SR has the highest flow rate, followed by DH, DD, and LH, which has the lowest flow rate. The difference in flow regimes between the wet season (higher flow) and dry season (lower flow) may explain the increased MP accumulation during the dry season in surface water, where the flow is slower and water exchange is reduced. In the dry

**Table 7.** MPs content in Oyster and Mudskipper biota samples at Can Gio Estuary collected in the dry and rainy seasons. MPs abundance showed the mean value and the standard error. Means were compared by Turkey HSD test at  $\alpha = 0.05$ , levels not connected by the same letter are significant different.

MPs Abundance	Bioaccumulation in dry season			Bioaccumulation in rainy season		
	Oyster (mean $\pm$ standard error)	Mudskipper (mean $\pm$ standard error)	Blanks (mean $\pm$ standard error)	Oyster (mean $\pm$ standard error)	Mudskipper (mean $\pm$ standard error)	Blanks (mean $\pm$ standard error)
Items/ sample(1)	4.5 $\pm$ 0.58 (ab)	1.0 $\pm$ 0.52 (cd)	0.33 $\pm$ 0.48 (d)	5.60 $\pm$ 0.52 (a)	2.60 $\pm$ 0.52 (bc)	0.33 $\pm$ 0.48 (d)
items/kg	7.5 $\pm$ 0.55	2.38 $\pm$ 1.84		9 $\pm$ 1.10	6.19 $\pm$ 1.94	
items/ individual	1.5 $\pm$ 0.16 (A)	0.2 $\pm$ 0.14 (B)		1.87 $\pm$ 0.14 (A)	0.52 $\pm$ 1.14 (B)	

(1) Each sample contained 5 individuals of mudskipper or 3 individual of oyster. Replication five times for each sample.

(2) One set of blank was done for each season. Blank was replicated six times for each season.

season, the accumulation of MPs in the water column at fast flow estuaries shows a significant influence of tidal currents, with a semi-diurnal tidal regime with two high and two low tides per day in this area. Further research is needed to quantify the interactions between season, tide, and flow rate on MP abundance at each location.

#### MPs between sample types

Figures 7, 8, and 9 compare MP data by sample type and by season. There was a difference in the number of MPs between water and sediment samples. The average range of MPs in both seasons was  $1.34 \pm 0.14$  to  $0.95 \pm 0.14$  (items/m<sup>3</sup>);  $4.22 \pm 0.46$  to  $2.44 \pm 0.46$  (items/L); and  $200 \pm 13.68$  to  $90 \pm 13.68$  (items/kg) for water surface, water column, and sediment samples, respectively. MPs in sediment samples were calculated in different units; therefore, they cannot be compared directly with MPs in surface water and water column (Zheng et al. 2021). The number of MPs in the water column samples was higher than in the water surface samples in terms of total MPs pieces and composition (post hoc test,  $p < 0.0001$ ).

This study found that there was a correlation between seasons and river level on the abundance of MPs in each sample type (surface, column, and sediment). Surface and sediment samples had higher concentrations in the dry season than in the rainy season for the smaller river branch, while there was

no difference between seasons for the large river estuaries. An inverse relationship was found for the water column, in which season influences the flow of the estuary more than the river branch (Figure 7, 8, 9). The explanation for this could be linked to the hydrological regime of the water system here. The hydrological conditions in the river system are one of the key driving factors for geological formation and habitats in this region. The hydrological regime here is characterized by an interaction between river flow from upstream and tidal currents from the East Sea; as well as seasonal variation over the hydrological year (Le 2011, Nguyen Huu 2000). Depending on the weather state, about 60 – 95 % of the aggregate flow velocity is due to tidal current (periodic variation over time and independent of weather). The weather-dependent flow component accounts for 10 – 40 % of the total flow velocity. The flow rate in the Can Gio region depends on the weather, ranging from 5 to 25 cm/s in the dry season, and can double in the rainy season (Nguyen Huu 2000).

In this study, the number of MPs in the water surface was over three orders of magnitude lower than in the water column in both seasons. However, the difference in the sampling methods employed could be one of the contributing factors. In a comparison study between grab (1 L) and net (335  $\mu$ m neuston net tow) sampling for MPs in the same water surface, Barrows and his colleague reported that number of MPs collected by grab



**Figure 10.** Two studied species, A) Sample of Oyster (*Saccostrea*), B) Sample of Mudskippers (*Periophthalmodon schlosseri*)

sampling was more than three orders of magnitude per volume higher than by net sampling (Barrows et al. 2017). In addition, the vertical transport of MPs in the marine environment is influenced by the MPs' density and the aggregates formation. As reported by Eo and colleagues (Eo et al. 2021), small high density MPs ( $> 1.02 \text{ g/cm}^3$ ) accounted for an average of 73% of total MPs in the continental shelf and deep sea waters of East Asia. Their study also reported that the aggregation of MPs could contribute to their vertical transport in marine water. These factors could contribute to the prevalent of MPs in the water column in this area, which would need further study.

#### MPs accumulation in the estuary biota

Pictures of the two studied species are shown in Figure 10. Table 7 shows the MPs accumulated in these estuary biota samples. MP content in oysters,  $1.6 \pm 0.3$  items/oyster digestive tract tissue, was higher than in mudskippers,  $0.36 \pm 0.37$  items/mudskipper digestive tract ( $p < 0.0001$ ). There was no difference between seasons in the MP content for both species. MP content in both species was significantly higher than in the blanks. This difference between species could be due to the species characteristics and their feeding style. Oysters catch their food by filtering water. Because of their specialized gill, oysters can catch food while respiring. During respiration, as water passes over the gills, food particles attach to the gill surface due to mucus generated by the cilia. The food particles that are too big to hold are removed by the water flow. Bivalves, such as oysters, are exposed directly to MPs present in the environment because of their extensive filter-feeding activity (Li et al. 2016). Mudskippers are capable of living both in the water and on the sediment surface in the mangroves. Due to the structure of their respiratory system, they can move long distances on land. Their specialized gills enable them to breathe air as long as they retain water inside their gill chambers.

Mudskippers eat small crustaceans like crabs, as well as snails and worms. A greater accumulation of MPs in oysters compared to fish was also found by other researchers (Danopoulos et al. 2020). Keerthika and colleagues studied MP accumulation in different species and reported that the abundance of microplastics in benthic species was higher than in pelagic species along the coast of India (Keerthika et al. 2023). The values found in this study were slightly lower than MP accumulation in the Dafeng River, China ( $2.2 \times 10^3 \pm 1.6 \times 10^3$  items/kg digestive tract tissue) (Liu et al. 2021), and in the Xiangxi River, China (0–1.5 items/fish digestion tract) (Zhang et al. 2017). Our results indicate that MP accumulation in local species is of significant concern for Can Gio Estuary and the Can Gio Mangrove Forest Conservation Reserve nearby.

#### Conclusion

Results from this study showed the presence of MPs in all sample types in both seasons at the Can Gio Estuary. Generally, MP abundances were higher in the dry season than in the rainy season. There were significant differences in MP content between sample types, with MPs in the water column being higher than in the water surface. The abundance and distribution of MPs were influenced by seasons and river flow regime. There was a difference in level of bioaccumulation, which was higher in oyster than in mud skipper. We suggest

further study on the correlation of hydrological factors and MPs accumulation to understand more about the formation and transport of MPs in this estuary region.

#### Author Contributions

Contribution to conception and methodology: Hoa T. Pham, Laura Michie, Simon Cragg. Collecting data and analysis: Tinh Q. Pham, Ngoc Pham, Hoa T. Pham, Phuc H. Trinh, Linh H. T. Nguyen. Drafting the article and revising it critically for important intellectual content: Tinh Q. Pham, Hoa T. Pham, Laura Michie, Simon Cragg. All authors contributed to the article and approved the submitted version.

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#### Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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