

Assessing microplastic dispersion from Saigon urban canals via Can Gio Mangrove Reserve to East Sea in Vietnam by Raman scattering microscopy

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Abstract

Plastic pollution is one of the significant environmental concerns due to the threefold increase in global plastic waste. Marine microplastics, including petroleum-based plastic pieces, synthetic and artificial fibers smaller than 5 mm, are ubiquitous in natural water environments due to the degradation of plastic products and wastes. This research aims to investigate the microplastic presence in urban and coastal environments in the Southeast regions of Vietnam by using Raman microscopy. As a result, most common plastics (PE, PET, PA, PP, PVC, PS and PMMA) were detected, and most of them were fibrous smaller than 500 μm . The total microplastics decreased gradually from the urban waterborne (up to 220 MPs/L) via Can Gio UNESCO Mangrove Biosphere Reserve (10 MPs/L) and to the East Sea (3 MPs/L), which reveals the potential role of the mangrove in reducing marine contaminants including microplastics. This study provides important insights about microplastic pollution in the Western Pacific Region, especially Saigon-Dong Nai river systems, supporting the useful data for natural water resources management.

1. Introduction

Plastic products are generally inexpensive, lightweight, and durable, bringing technological and medical advances, energy savings, and other societal benefits. Therefore, the plastic industry has increased the global manufacture substantially, up to nearly 370 million tonnes in 2019 (PlasticsEurope 2020). Mismanaged plastic waste has introduced 8–12.7 million tonnes of plastic (PP, PE in prevalence) in the ocean every year (Scalenghe 2018). The top polluting rivers globally are primarily located in Asia, accounting for 67% of the global total. Vietnam is in the world-top countries with the estimated amount of plastic waste discharged into the sea from 0.28 to 0.73 million tons/year (Le 2019), and ranked fourth in the world about plastic waste mismanagement (Jambeck et al. 2015). Southern Vietnam featured an interlaced system of rivers, streams and canals with asymmetric semi-diurnal tides. Ho Chi Minh City (HCMC, Saigon) is the most populous and largest economic center of Vietnam. In 2014, about 8,175 tons of municipal solid waste were generated daily, textile and plastic components accounted for 5–7.2% and 16–25%, respectively (Verma et al. 2016). Saigon (SG) River flows to HCMC, forming main canal systems – Nhieu Loc-Thi Nghe (NL-TN), Te (KT), Đòì (KĐ), Tau Hu (TH), Tan Hoa-Lo Gom (LG). At least 2000 metric tons of floating debris are collected every year on the main urban canals of Saigon (Kieu-Le et al., 2016). Saigon River merging with Đong Nai River flows through Can Gio Mangrove to Ganh Rai Gulf of the East Sea (South China Sea). Can Gio, a coastal district of HCMC, is the first Mangrove Biosphere Reserve of Vietnam designated by UNESCO in 2000. It functions as a natural water filter for marine environment by remaining and diluting soluble pollutants in rivers from the city. The reserve is divided into three zones: (1) core zone (4,720 ha) with few households and strict forest protection; (2) buffer zone (37,340 ha) where traditional exploitation in rivers is unregulated; (3) transition zone (29,310 ha) where intensive aquaculture (oysters, shrimps, clams, etc.) and tourism are along the shoreline (Cormier-Salem et al. 2017).

Plastic items tend to be broken into small particles with a wide range of shapes and sizes under chemical weathering, photo-degradation, physical and biological reactions. Annually between 0.8 and 2.5 million tonnes of microplastics (MPs), two-thirds of them are synthetic fibers released during washing, erosion of tyres while driving (E.-L. Ng et al. 2018). According to the size, they are classified as mesoplastics (> 5 mm), large microplastics (1–5 mm), small microplastics (≤ 1 mm) and nano plastics (< 100 nm) (Mai et al. 2018). Plastic debris causes an aesthetic loss of urban landscape, poses a hazard to aquatic wildlife and human maritime activities. Nearly 700 marine species such as dolphins, sharks, crocodiles, crabs and invertebrates have been reported to be affected by

microplastics. “Ghost fishing” from fishing nets left on the beach due to accidental or deliberate fishing activities could entangle turtles, coral reefs, other valuable creatures, and even human divers, resulting in losses to commercial fisheries and lethal dangers of marine entertainment (Gregory 2009). Some species specifically select plastic debris since they mistake similar-size microplastics for food (Botterell et al. 2019). The uptake of microplastics results in physical conditions such as bowel obstructions (Udayakumar et al. 2021), reduced food intake, behavior changes, and chemical effects such as inflammation, hepatic stress, reduced reproductive output due to plastic additives (Auta et al. 2017).

The microplastic assessment is still a big challenge owing to their homogenous distribution. Moreover, the inter-study comparison on marine microplastic abundance is usually impossible due to the lack of standardization between sampling and analytical methods. The general principle is the filtration process which is performed by a manta trawl (on-site filtration) or a vacuum filtration system (bulk sampling). Net sampling is preferable for large-scale surface water sampling (Mai et al. 2018) and can simplify the analysis procedure as micro-particles are all kept in the net. Nonetheless, a manta trawl system is expensive, and the result depends on the net size. On the other hand, bulk sampling is more advantageous for point sampling (Karlsson et al. 2020). As there are no size limitations in the sampling, a wide range of size fractions, from large to smaller microplastics, can be identified. A typical workflow for microplastic analysis comprises physical and chemical characterizations. Physical identification includes a microscope, and if necessary, combined with a melting test for > 50 µm microplastics (De Witte et al. 2014). The visual sorting is based on microplastics’ morphology observed under a dissection microscope with or without stained with Rose Bengal (Kosuth et al. 2018), a fluorescence microscope after dyed with Nile Red (Shim et al. 2016), or a scanning electron microscope (SEM) (Fries et al. 2013). Although the Nile Red method is highly sensitive, biogenic materials such as lipids and chitin will interfere with the plastic detection, and plastic types may give similar color signals (Erni-Cassola et al. 2017). Hence, the visual methods should be combined with a spectrometer. FT-IR spectroscopy is a convenient tool to determine marine microplastics (Song et al. 2015, Cincinelli et al. 2017). Raman spectroscopy based on the interaction of molecules with photons in monochromatic light has recently attracted more attention (Schymanski et al. 2018, Gillibert et al. 2019) due to the non-destructive feature, better resolution, wider spectral coverage and lower water interference (Ribeiro-Claro et al. 2017). It can replace FT-IR or be used with FT-IR to obtain the best results (Käppler et al. 2016). In addition, thermal analysis such as TED-GC/MS, pyrolysis-GC/MS has been employed with high reliability (Fries et al. 2013, Hermabessiere, 2018).

Saigon-Dong Nai basin is one of the main river systems of Vietnam, and there are several studies on its plastic pollution. There are four main river systems through Can Gio Mangrove: (1) Soai Rap (Western border with Mekong Delta), (2) Dong Tranh (Northern border with Dong Nai), (3) Thi Vai river (Eastern border with Vung Tau) (4) and Long Tau, which flows to Ganh Rai Gulf. However, the previous studies only focused on microplastics in the Saigon River using the FT-IR method, and the presence of microplastics in UNESCO Can Gio Biosphere Reserve as well as Ganh Rai Gulf have not well reported in any papers. This article aims to prove the specificity of micro-Raman spectroscopy and employ the method in investigating microplastic pollution from Saigon urban canals to Can Gio Mangrove and eventually to the East Sea.

2. Materials And Methods

2.1. Reagents and equipment

The powdered standard plastics were purchased from Goodfellow (UK): low-density polyethylene (LDPE) with an average particle size of smaller than 300 microns; polyethylene terephthalate (PET) 300 μm , un-plasticized polyvinyl chloride (uPVC) 250 μm , poly(methyl methacrylate) (PMMA) 85 μm , and nylon-6 (PA-6) particles 15–20 μm in size. In addition, the raw pure plastic particles (diameter of 3 mm) of PP, HDPE and PS were supplied by Vietnamese plastic production companies.

Filtered deionized water (FDW) was prepared by filtering deionized water through 0.4-mm filter membrane (Sigma Aldrich) with the support of a vacuum filtration system before use. Whatman GF/A glass-fibre filter paper (diameter 47 mm, pore size 1.6 μm) was used to extract microplastics.

A ZEISS Stemi 508 Stereo Microscope with 8:1 Zoom was firstly used to observe the whole area of the GF/A membrane and count the total number of particles. Every single piece was then analyzed in viewing and Raman modes with the XploRA Horiba Raman One 532 nm integrating with optical parts of Olympus (Horiba Scientific, USA).

2.2. Study areas and sampling methods

The sampling locations were marked on the map (Fig. 1) (see detailed description and coordinates in **Table S1**). Bulk sampling was used in the same way in several previous studies (Lahens et al. 2018, Su et al. 2016, Lusher et al. 2014) from February 2020 to March 2021. At each location, at least 5 liters of water was collected using Wildco horizontal alpha water sampler and bucket in the middle along every canal system in the center of Ho Chi Minh City (Saigon urban canals); randomly in transition, buffer and core zones of Can Gio mangrove from the boat; and in the sea (ES1, ES2, ES3 and ES4) from the canoe.

2.3. The workflow for marine microplastic extraction and analysis

Microplastics were extracted from the water samples by using the procedure of Khuyen et al. 2021b. With the aid of a vacuum pump, a particular volume of water samples was directly filtered through a GF/A membrane until the membrane became brownish yellow. Visible items on the filter membranes were picked up, cleaned with filtered deionized water (FDW), and stored for microplastic observation. All materials were immediately washed off with FDW, and the final volume of supernatant containing microplastics was reduced to only 100–200 mL. The supernatant was then digested with 30% hydrogen peroxide at 70–80 $^{\circ}\text{C}$ until the solution was discolored, and the mixture was incubated at room temperature overnight. Finally, the mixture was filtered and microplastics deposited on the filter membranes were analyzed with Raman microscope.

2.4. Quality control

There was a field control sample at each sampling location. Glass bottle containing 1 liter of FDW was opened and placed at during the sampling collection. The air control samples were clean filter membranes on glass Petri dishes around the working area during sampling handling, vacuum filtering, and microplastic analysis. The water control samples were bottles containing 500 mL FDW. All the control samples were directly analyzed to observe whether contaminating microplastics found in the actual samples. As a result, no microfibrers were found, showing that fibers found in the study were not from clothes worn in the laboratory and during the sampling.

2.5. Statistical analysis

The number of pieces was counted manually and classified by polymer type, shape, and color. The microplastic abundance in samples was expressed as items per liter of water (MPs/L), and the results were evaluated for statistical homogeneity by analysis of variance (ANOVA). Turkey's multiple comparison test was used to analyze the mean differences between sampling locations in Saigon canal systems, between locations in Saigon with Can Gio, and with the East Sea. All statistical analyses were performed at a 95% confidence interval on SPSS v22 software.

3. Results And Discussion

3.1. Combined qualification and quantification of microplastics by Raman microscopy

Visual identification: The viewing mode includes two objectives: 10x and 50x. The larger objective allowed to zoom powdered particles in the case of PA and PMMA. However, the images were not always clear. Thus, the shape, color and size (μm) were observed at 10x. The final size was calculated depending on the shape. It was expressed as length for microfibers, and the sum of width and length for non-fibrous forms.

The morphology of debris under the microscopic mode could be used to quantify the microplastics. Some criteria described by Zhao et al. 2016 were applied to distinguish microplastic fibers (clothing, fishing nets, for instance) from non-plastic fibers (organic, or cellular structures, Fig. 2a). Microplastic fibers were (1) clear and homogeneously colored, (2) soft and three-dimensional bendable Fig. 2c; (3) equally thick, not segmented and not tapered towards the ends. Fibers especially dark-colored ones meeting above requirements but having complicated spectra were called "unidentified group". Moreover, the lengths of some fibers were close to the widths, and these cases were recorded in the shape of "bars or sticks" instead of particles as suggested by Enders et al. 2015. Non-fibrous shapes included particles with either spherical or aggregate of spheres, fragments (particles with jagged edges as a signal of fragmentation), and films with square or rectangle images. However, the film category always accounted for a small percentage, mainly because they were broken down into threads and filaments, thereby classified into fiber categories (Chubarenko et al. 2016). The other groups are foams, pellets (granules with the shape of a cylinder or a disk manufactured as a raw material of plastic goods).

In addition to shapes, colors can be a preliminary tool to guess the plastic type, behavior and origin of microplastics. Clear and transparent particles have been ascribed to polypropylene (PP) (Ismail et al. 2009), while black represented the sorption of PAHs and PCBs on PP and polystyrene (PS) (Frias et al. 2010). White pieces were assigned to polyethylene (PE) (Ismail et al. 2009), and their film or sheet shapes suggest they come from shopping bags, agricultural and food packaging films (Zhu et al. 2018). Colors can also indicate the weathering and decomposition degree, and residence time at the seawater surface. The discoloration can be caused by long exposure of UV-light (translucent color) (Kunz et al. 2016, Crawford and Quinn 2017). In other words, the discoloration (yellowing) is a result of oxidized PCBs adsorbed on plastic resins in the environment (Endo et al. 2005).

Spectroscopic identification: The Raman spectrometer with a 532 nm excitation laser and a charge-coupled device (CCD) detector was used to measure spectra of each item at x50 objective in an integration time of 15s and a grating of 900 gr/mm in a range wavelength from 50 to 3600 cm^{-1} . In practice, plastic additives and organic pollutants such as PCBs, PAHs adsorbed on microplastics' surface made the spectra more complex. The sample

treatment was performed on all polymer standards. The results showed their morphology and Raman spectra were not affected by peroxide oxidation. Thus, the peak shifts of sampled microplastics were mainly caused by the degradation in the environment. Nevertheless, as can be seen in Table 1, vibration wavenumbers of functional groups in pure plastic molecules could be recognized in the sampled microplastics' spectra, which pointed out the high specificity of Raman spectroscopy in identifying chemical composition of most common plastics.

Table 1. Raman band assignments of standard plastics and sampled microplastics

Polymer	Raman bands (cm ⁻¹) of sampled MPs	Raman bands (cm ⁻¹) of plastic standards	Bond assignment
Polyethylene (LDPE)	2928.90	2884.50	-CH ₂ - stretch (d)
	1442.63	1441.90	-CH ₂ - wag (d)
	1293.13	1296.25	-CH ₂ - twist (d)
	1172.35	1124.36	C-C stretch (d)
	1069.69	1060.97	C-C stretch (d)
Polypropylene (PP)	2886.50	2889.14	-CH ₃ stretch (c)
	1451.79	1458.89	-CH ₂ - deformation (b)
	1325.16	1328.97	C-C stretch (c)
	1150.51	1153.82	-CH ₃ rock (c)
	805.23	808.67	-CH ₂ - rock (b)
Polyvinyl chloride (PVC)	2892.78	2852.09	C-H stretch (i)
	1436.72	1430.19	-CH ₂ - bend (i, e)
	1360.33	1319.11	unknown group (i)
	693.92	632.64	C-Cl
Poly-ethylene terephthalate (PET)	1753.59	1725.34	C=O stretch (g, h)
	1602.3	1614.52	 C=C (h)
	1302.58	1288.21	C-C
	846.1	855.40	C=C (g)
	693.92	628.46	 (g)
Polystyrene (PS)	1612.87	1604.24	phenyl ring stretch (a, b)
	1015.03	1001.75	C-C in-plane ring deformation +, C-H out-of-plane deformation (a)
	622.43	621.10	in-plane ring deformation (a, b)
Poly (methyl methacrylate) (PMMA)	1679.85	1723.71	C-O-C symmetric stretch (a)
	1512.10	1447.51	-CH ₂ - deformation (b)
	1154.13	1181.31	C-C-C-C stretch (b)
	835.31	808.67	C=O stretch (a)
Polyamide-6, nylon-6 (PA-6)	2895.67	2900.2	-CH ₂ - stretch (e)
	1680.05	1633.39	unknown group (e)
	1440.22	1441.9	-CH ₂ - (e, f)
	1383.02	1430.49	-CH ₂ - bend (e, f)
	1094.93	928.73	-CH ₂ - twist (f)

(e) Gündoğdu 2018, (f) Milani 2015, (g) Käßler et al. 2015, (h) Alexiou et al. 2020, (i) Solodovnichenko et al. 2016

3.2. Microplastic pollution in the freshwater at Saigon urban canals

Figure 3 showed the uneven distribution of microplastics in locations of Ho Chi Minh City. Microplastic amount was highest in residential areas, 45.89 ± 24.04 MPs/L in Bridge No. 1 (SG1 on the map, Fig. 1) where floating rubbish from NL-TN canal was gathered, varied from 44.33 to 58.57 MPs/L in Tau Hu Canal (SG8, SG9, SG1, SG11) belonging to Districts 6, 7, 8 and Tan Binh. Microplastics were found with a noticeably high abundance in some samples at the locations where the water environment was concentrated (dark colour) with a higher number of visible floating garbage such as food boxes, clothing pieces and nylon bags. Among the repetition times, there were replicates obtaining very high abundance of plastic debris, for instance, 160 MPs/L in Bridge No. 1 (SG1, Fig. S1a), 110 MPs/L in Letter Y Bridge (SG10), 125 MPs/L in Lo Gom canal (SG10), and 220 MPs/L in Kenh Te Bridge

(SG12). There was a dramatic decrease in the microplastic amount from the pollution sources to Saigon River estuaries, for example 23.92 ± 19.23 MPs/L in Bach Đang Wharf (SG7), and 27.33 ± 6.23 MPs/L in Khanh Hoi Bridge (SG15). Indeed, Turkey's multiple tests showed significant statistical differences between Kenh Te Bridge and Khanh Hoi (Sig.=0.005, **Table S4**), and Bach Dang Wharf (Sig.=0.021, **Table S4**). There were no statistical differences between other locations.

These locations are grouped into four canals according to geographical division of the Government. Nhieu Loc-Thi Nghe is a separate system inside HCMC flowing to Saigon River, while other canal systems connect HCMC with neighbour cities. Ben Nghe Canal (3.1 km) originates from Lo Gom-Tau Hu Canal system (9 km), Te Canal (KT, 4.5 km) is the continuation of Doi Canal system (8.5 km). Doi-Te, Ben Nghe-Tau Hu canal basins join at the position of Nguyen Van Cu Bridge before flowing to Saigon River. Regarding the composition (Table 2), the percentage of plastic types were similar at close locations belonging to each canal system. In general, nylon was the most abundant component, followed by polyester fibers. PE was also the second most frequently sorted in Ben Nghe and Te basins. In Nhieu Loc and Lo Gom basins, PE and PP were identified at the same proportions. Polystyrene was found with a higher percent (around 12%) in the study of Emmerik et al. (2019) but made up small percentages in our study. In fact, many fragments of PS food boxes were seen floating on surface waters while sampling, but they were regarded as macroplastics. Small-size particles in black colour had only Raman band at 1000 cm^{-1} of PS, which were not enough to assign them as PS microplastics. Physical processes, particularly wind blowing and water mixing influenced sinking behavior of plastic debris, strongly affecting the presence of PS micro-size classes (< 2 mm) (Frère et al. 2016).

Table 2
Compositions of microplastics extracted from main Saigon urban canals

Plastic composition	Nhieu Loc – Thi Nghe (NL)	Tau Hu (TH)	Ben Nghe (BN)	Te Canal (KT)
Total microplastics (MPs/L)	35.58 ± 4.75	45.19 ± 10.30	33.83 ± 10.54	43.41 ± 7.93
PE (%)	14.35	12.23	14.68	17.41
PP (%)	13.48	12.66	11.51	11.34
PET (%)	21.74	18.34	8.73	7.283
PVC (%)	5.65	6.11	11.51	4.86
PS (%)	3.04	2.18	4.76	1.21
PA (%)	22.17	23.58	14.68	17.41
PMMA (%)	1.31	0.87	0.79	1.21
Unidentified (%)	18.26	24.02	33.33	39.27

Nhieu Loc-Thi Nghe canal system plays an important role in the city's activities during the foundation and development. This 8.7-km long canal flowing through seven districts was the cleanest canal at the beginning of the reclamation of land in Southern Vietnam. It became the dirtiest canal from the 1970s mainly due to the discharge of solid waste and wastewater from the slums on both sides of the banks. The water quality in this

canal has been significantly improved since 1993. It is now the cleanest canal in the city, thanks to the largest canal clean-up programs (HCMC Environmental Sanitation Project) funded by the World Bank and the City Government (Kieu-Le et al. 2016). Nonetheless, plastic pollution is still a concerning problem because plastic wastes can come from different sources such as direct dropping litter on land or at sea, blowing, leaching from landfills, and losses during the transport. According to a statistical data of Barnes et al. 2009, a large proportion of 40–80% of plastic garbage is from carrier bags, packaging, footwear, cigarette lighters and other domestic items. Indeed, the fact in all urban canals is floating debris which comes from discarding wastes of tourists and residents, and natural vegetal, mainly water hyacinths drifted from Sai Gon River during high tides. The floating debris life cycle is specific to each canal and not involved in solid waste management. The floating debris collection is very active in Nhieu Loc – Thi Nghe canal, conducted every day from 6 am to 6 pm. Indeed, the average values showed the lowest abundance of microplastics in this canal (Table 2), which is similar to the study of Lahens et al. (2018). The water quality of KT and TH-BN canal basins has been improved to some extent thanks to HCMC Water Environment Improvement Project funded JICA. Nonetheless, TH and LG canals flow along with the highest populated districts with many slum areas along the canals. The mismanaged wastewater management and ineffective floating debris collections in these districts led to higher pollution compared to Nhieu Loc canal (Kieu-Le et al. 2016).

The average of MPs in our study (38.45 ± 24.93) was two orders of magnitude lower than the range of total microplastics reported by Lahens et al. 2018 (172–519 fibers/L). The noticeable differences can be explained by different time periods, sampling techniques and analysis methods rather than the levels of plastic pollution in the studied regions as proposed by Enders et al. 2015. A few bundles of green fibers (Fig. 2a) were detected in the state of twisted together, thereby considered as “non-microplastics” due to unclear shapes. Moreover, we found a large number of black particles whose images and spectra were difficult to identify. Indeed, Raman analysis of black particles resulted in a considerably higher rejection rate, as suggested by Lenz et al. 2015. Hence, only black items with clearly homogenous shape and identifiable structural characteristics were included in microplastic data, consequently small percentages (1.58–6.7%) of black color.

3.3. Spatial distribution of marine microplastic from Can Gio Biosphere Reserve to the East Sea

The downstream of Saigon River merges with Dong Nai River to form Nha Be River and divides into two branches: Long Tau and Soai Rap Rivers, flowing to the East Sea (South China Sea) through Can Gio Mangrove. Plastic pollution sources, including direct dropping litter on land or at sea, blowing, leaching from landfills, and losses during the transport, are mainly in the transition zone (from CT1 to CT8 on the map). Nevertheless, its total microplastics (10.45 ± 3.67 MPs/L) are similar to the buffer zone (from CB1 to CB5 on the map) (10.75 ± 3.13 MPs/L), where human activities are more restricted. The geographical position is proposed to be the main reason. The transition zone consists of outer areas that are strongly affected by coastal features such as winds, waves, tides, river runoff which makes microplastics dispersed and diluted unevenly (Lusher et al. 2014). Moreover, there are no clear geographical maritime boundaries between the zones because adjacent areas share the local canals and rivers.

Figure 4 indicates the distribution of plastic types in the zones of Can Gio reserve. PE was the most abundant type in the transition and core zones, and almost all of them were fragments and particles. PET was the largest group in the form of fibers (35%) and fragments (35%) in the buffer zone but was the second dominant, mainly in fibrous form (53.57%) in the transition zone and in both fibers (31.25%) and particles (31.25%) in the core zone. Fibers

were found to be abundant in the transition zone, especially coastal locations. Can Gio 30 April Beach is the most attractive recreational beach, which is considered as a vital pollution source, especially polyamide fibers (Khuyen et al. 2021a). Fishing is the main income source of the local people, especially in Can Thanh. Zhu et al. (2018) proposed that the origin of blue nylon fibers is cables used in fishery activities. Moreover, fibers could originate from shipping (Lusher et al. 2015), especially at the intersection of main rivers – Dong Tranh and Long Tau, where large ships circulate every day. In addition to plastics' density at this location, buoyancy effects of marine plastics in the water column were mediated by a high level of surface mixing caused by boat movement and seawater turbidity (Lusher et al. 2014). As a result, PET and PA dominated (26.53%), following by PP (20.41%), and PE (10.2%).

In other words, microplastics were observed but with less number (7.48 ± 1.28 MPs/L) in the core zone (from CC1 to CC3 on the map) although human's domestic activities are strictly prohibited. This proves microplastics can be detected in areas far from pollution sources, and their abundance would depend on the proximity to the urban sources. Microplastics, therefore, could be released from outer regions, floated under the natural forces of water flows and accumulated in convergent zones (Lusher et al. 2014). The sediment of mangrove forest can be a suitable environment for microplastic storage because we found some white fragments and blue fibers similar to ones in the seawater. The debris is difficult to detach from the sediment due to sticky characteristics of the sediment. Furthermore, pollutants retained in the core zone would be difficult to return to the marine environment because the waves are attenuated, and the water surface here remains calm (Mazda 2009). Some studies reported the presence of microplastics in the mangrove sediment and roots (Li et al. 2019), but the role of mangroves as a filter of plastic debris for the seawater needs more investigations.

Figure 5 demonstrates remarkable changes in the relative abundance of microplastics from Ho-Chi-Minh City (HCMC, Saigon urban canals/rivers) through Can Gio Mangrove (Can Gio seawater) to the East Sea (Soai Rap Estuary, from Long Tau Estuary to Ganh Rai Gulf, and White Tiger Oilfield). Mangrove is a special soil accumulating carbon, nutrients and sediments as "enhancer of sedimentation" (Valiela and Cole 2002). Sediments deposited into mangroves come from allochthonous sediments (external sources like terrestrial or oceanic sources), and autochthonous sources (re-suspended sediments) (Adame et al. 2010). Microplastics can be transported from the oceanic environment to the mangrove and accumulated in the sediment layers in a similar way as in the aquatic environment (Auta et al. 2017). This is considered one of the main reason for a low number of microplastics within Can Gio reserve. Indeed, the decrease in number of plastic debris highlights the feasible role of the mangrove in retaining contaminants including microplastics.

The Turkey's test showed a statistical difference in total MPs between Binh Khanh ferry (CT1) with Ganh Rai Gulf (ES3) (Sig.=0.03, **Table S3**), and with Soai Rap estuary (Sig.=0.03, **Table S3**); Turkey's test showed a statistical difference in total MPs between Binh Khanh ferry with Ganh Rai Gulf (Sig.=0.03, **Table S3**), and with Soai Rap estuary (Sig.=0.03, **Table S3**); between Rach Don Bridge (Can Gio buffer zone, CB1) and Ganh Rai Gulf (Sig.=0.014, **Table S3**). The comparison tests indeed showed some statistical difference between Saigon urban canals with Can Gio Mangrove and the East Sea, for example, Bui Huu Nghia Bridge (SG5) with transition zone (C1-Sig.=0.01, C2-Sig.=0.038, C3-Sig.=0.018, **Table S4**), with downstream of Soai Rap River (ES2) (Sig.=0.004, **Table S4**), downstream of Long Tau River (ES1) (Sig.=0.036, **Table S4**), Ganh Rai Gulf (ES3) (Sig.=0.001, **Table S4**), and White Tiger Oilfield (ES4) (Sig.=0.011, **Table S4**).

The pollution sources concentrate in HCMC, and therefore the MPs amount was highest (nearly 40 items/L) and decreased gradually to the estuary areas. The number of microplastics in the Saigon river-estuarine system of our

study falls into the range of concentrations reported by Strady et al. (2020), which was 22 to 251 MPs/L. At the intersection of HCMC and Can Gio (Binh Khanh Ferry), the microplastic concentration decreased to below 20 MPs/L. The average abundance of MPs was around 10 MPs/L within Can Gio reserve, which was in the range of microplastics in Three Gorges Reservoir in China (1.597–12.611 MPs/L) (Di and Wang 2018), Taihu Lake in China (3.4–25.8 MPs/L) (Su et al. 2016). This comparison could be made owing to the similarity of the position of Can Gio and Taihu in terms of geographical location and human activities. Nevertheless, Taihu Lake has become one of the most severely polluted lakes in China due to the development of the local economy and industry, presence of three wastewater treatment plants, but, in contrast, Can Gio is strictly protected by the government and UNESCO. Microplastics have eventually reached the East Sea, but with a smaller amount, from 3.1 ± 1.6 to 4.7 ± 3.2 MPs/L.

The total microplastics in Ganh Rai Gulf was much smaller than Jinhae Bay, South Korea (88 ± 68 MPs/L) (Song et al. 2015b), but equivalent to Hudson River (USA) to some extent (0.625–2.45 fibers/L) (Miller et al. 2017). Microplastics in Ganh Rai (ES3) are predicted to come from terrestrial and sea-based sources. The later source is supposed to maritime transport (Sheavly et al. 2015, Hasnat and Rahman 2018), because the sampling sites are around the intersection of cargo ships, vessels carrying imported-exported fuels and gases. However, it is still well understood which is the main reason for the presence of plastic debris in this area. According to Browne (2015), the source of maritime shipping is much smaller than land-based sources. In fact, microfibers accounted for larger percentage than micro-parciticels (**Table 3**), which is attributed to textile sources from the mainland. All of investigated plastic types were encountered from the ocean surface to the depths of 3 meters in the sampling locations of Ganh Rai Bay. PE dominated on the sea surface, whilst PA, PET, and PVC were predominant in the sub-surface samples, 2 meters and 3 meters, respectively. They are subjected to distribute more in the deeper seawater levels since their densities (up to 1.14, 1.3–1.5 and 1.15–1.70 g/cm³, respectively) were higher than seawater (1.023 g/cm³ in measured average) (Crawford and Quinn 2017; Weber et al. 2018; Issa and Kandasubramanian, 2021). PS was found in the sub-surface seawater with a higher abundance despite its similar density to the seawater. Hence, it can be seen that the vertical distribution of microplastics depends on not only the density of virgin polymer. Their distribution is also affected by the disturbance caused by the actions of internal wave and oceanic circulation modes in the seawater (Wang et al. 2016) since any abrasion, cracking and pitting of the surface can increase the density (Crawford and Quinn 2017).

As seen in **Table 3**, the majority of microplastics were fibers with various size fractions. There was a surprising similarity in their colours in most environments. White was always most predominant in all shapes of plastic types, particularly 18.1% for fibers. Pink (red), blue, purple, gray were popular colors of fibers (Fig. 2), 15.7%, 14.4%, 12.4% and 10.2%, respectively. Black accounted for 7.6% of fibers. However, the black was seen with purple or blue instead of completely black as activated carbon. These colours were also commonly found in fibers in the Northeast Atlantic Ocean (blue, black, red) (Lusher et al. 2014), Jinhae Bay, South Korea (green, blue, red) (Song et al. 2015b), Qatar (blue, white) (Castillo et al. 2016), Hudson River-USA (blue, black, red) (Miller et al. 2017). The results showed the distribution of plastic types was not much different by the depths in the water column. PE dominated in the surface samples, PET and PA were predominant in the sub-surface samples. PE, PP and many PET fragments were attributed to breakdown products of plastic items, especially single-use water bottles and tableware. In Ganh Rai, Soai Rap and White Tiger seawater, pink fibers were dominated by PET (Fig. 2e), while blue was more frequently observed in PA fibers (Fig. 2d). In the water of Saigon canals, some parts of several coloured fibers appeared in brown, which showed the contamination of coloured substances on the surface of microplastics in the environment.

Table 3. Comparisons of parameters of microplastics in Saigon urban canals, Can Gio Reserve and East Sea

Parameters	Saigon urban canals (Saigon River)	Can Gio Reserve	Downstream of Soai Rap River	Ganh Rai Gulf (East Sea)	White Tiger oilfield (East Sea)
Plastic compositions	PA > PET > PE > PP > PVC > PS > PMMA	PET > PE > PA > PP > PVC > PS > PMMA	PE > PP > PET > PA > PVC = PS > PMMA	PET > PE > PA > PP > PVC ≥ PS > PMMA	PET > PA > PP > PE > PVC > PS = PMMA
Common shapes (%)	Fiber: 35.4 Fragment: 20.6	Fiber: 27.7 Fragment: 34.2	Fiber: 31.3 Particle: 21.8	Fiber: 42.4 Particle: 20.14	Fiber: 44.1 Particle: 26.5
Common colors	white > gray > brown > green > pink	white > gray > blue > pink > brown	white > pink > blue > purple > yellow	white > blue > pink > gray > green	white > gray > pink > blue > purple
Size range (µm)	Fiber: 14.1–561.2 Non-fibers: 15.7–188.4	Fiber: 25–60 Non-fibers: 14–53.4	Fiber: 19.7–58.3 Non-fibers: 21.9–94.5	Fiber: 25–436.9 Non-fibers: 23.5–265	Fiber: 36.1–184.7 Non-fibers: 22–92

Fibers were also commonly encountered in Can Gio reserve. However, their percentage (21–22%) was still lower than that of fragments (33–36%) (**Table 3**). The size of plastic debris in this mangrove varied from 15 µm to 53.38 µm, so smaller than those in Saigon urban canals (from 15 µm to 197.5 µm). Similarly, microfibers were only 60 µm maximum (53.43 µm in average) in the mangrove but were up to 561.23 µm (122.76 µm in average) in Saigon canal systems. The shape of fibers in locations of Can Gio and East Sea (Fig. 2b, c, d **and e**) looked similar which is single colored fibers, which suggests that microfibers have dispersed in the marine environment. Differently, bundles of microfibers (Fig. 2a) were quite often found in only Saigon urban canals. The small size fraction (25–50 µm) is quite common in the mangrove systems as also reported in mangrove sediment of Maowei Sea (Li et al. 2019) and in Singapore's coastal mangrove ecosystem (Nor and Obbard 2014). The high percentages of small plastic fragments revealed that mangrove systems might accelerate plastic decomposition and accumulation. This, therefore, can promote harmful effects of plastic debris on the mangrove benthos to some extent.

Conclusion

Investigating microplastics in the aquatic environment is a time-consuming procedure of multiple steps, including sampling, separation, qualification, and quantification. Raman scattering microscopy is a powerful tool to assess the microplastics in a water matrix with high specificity and low interference of water molecules. This study found that microplastic concentration decreased gradually from the urban waterborne (30–250 MPs/L) to estuaries (10–20 MPs/L) and to the sea (3–5 MPs/L), which highlights the dispersion of microplastics in the marine environment. The data showed the variability in morphology and plastic composition of marine microplastics. Polyethylene, polyester and polyamide were predominant polymers of marine debris in the estuary location of Southern Vietnam. The findings indicated that the presence of marine plastic debris was associated with not only proximity to land-based sources, population density, but also natural water circulation in the sea. Nonetheless, it is

a practical challenge to determine the exact sources and fate of microplastics in the coastal and marine environment because they are distributed randomly throughout the water column, especially in the sea surface microlayer. Finally, the accumulation of very small microplastics in fibrous and non-fibrous forms in the seawater likely caused by the degradation of plastic waste warns the risks of microplastic pollution in UNESCO Can Gio Mangrove Biosphere Reserve.

Declarations

We confirm that all authors of the manuscript have no conflict of interests to declare. We confirm that the manuscript is the authors' original work under the scholarship of Vo Thi Kim Khuyen (the first author). We confirm that the manuscript now submitted is not copied or plagiarized version of some other published work. The manuscript has not received prior publication and is not under consideration for publication elsewhere. We declare that the paper shall not be submitted for publication in any other Journal or Magazine until the decision is made by journal editors.

AUTHOR CONTRIBUTIONS

I confirm that all authors listed on the title page have contributed significantly to the study conception and design, and agree to its submission. Vo Thi Kim Khuyen developed the protocol and microplastic analysis workflow, collected the interview data, conducted the experiments and wrote the first draft of the manuscript. Vo Thi Kim Khuyen, Le Hung Anh designed the sampling plans and sampling map. Vo Thi Kim Khuyen, Dinh Vu Le structured the manuscript, analyzed the data and performed statistical analysis. Christina Dornack, Axel Fischer supervised, evaluated the experimental procedures and results, reviewed and edited the manuscript. All authors discussed the results, read and approved the final manuscript.

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Figures

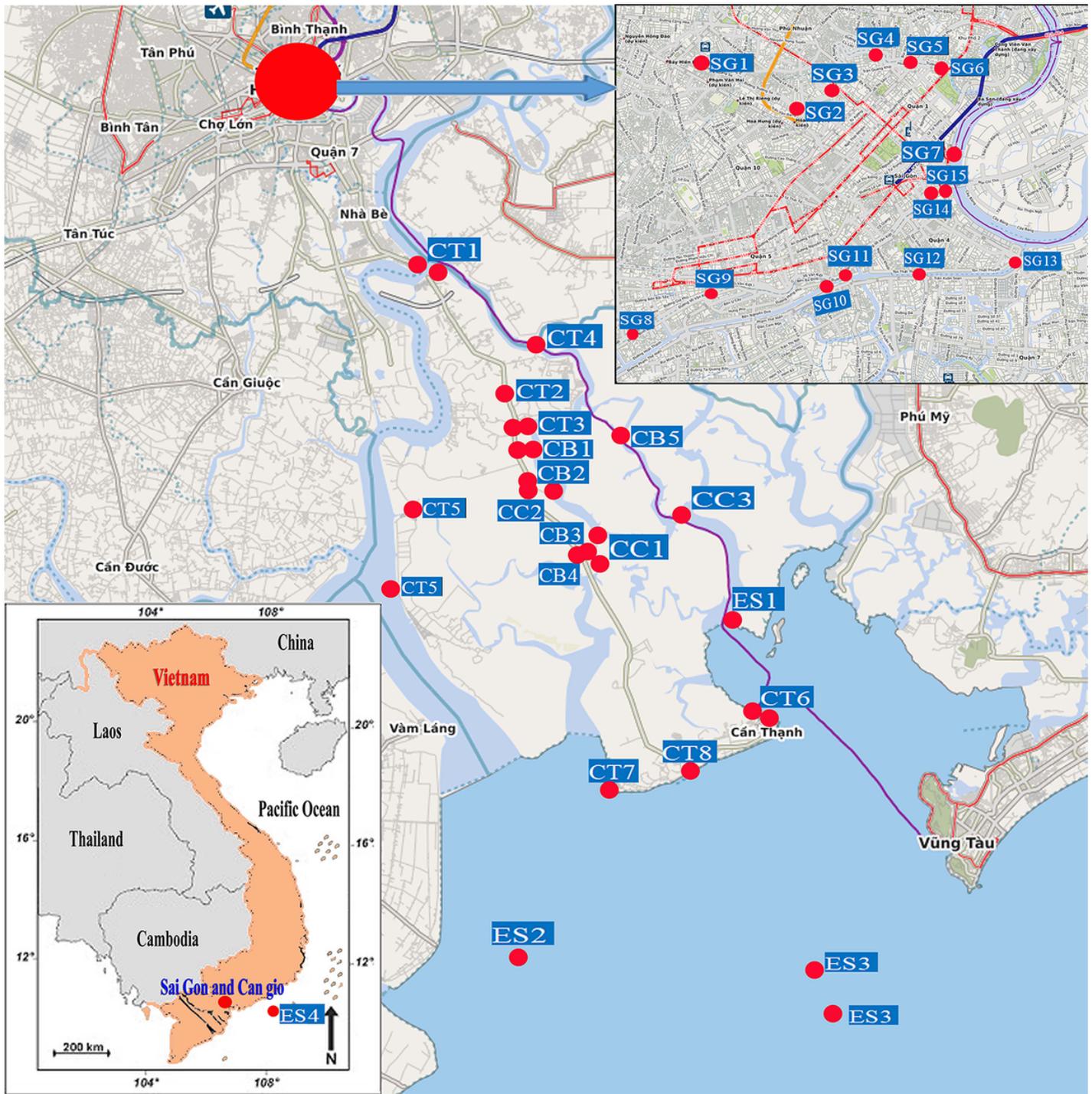


Figure 1

Geographical map of Vietnam, study areas and sampling locations

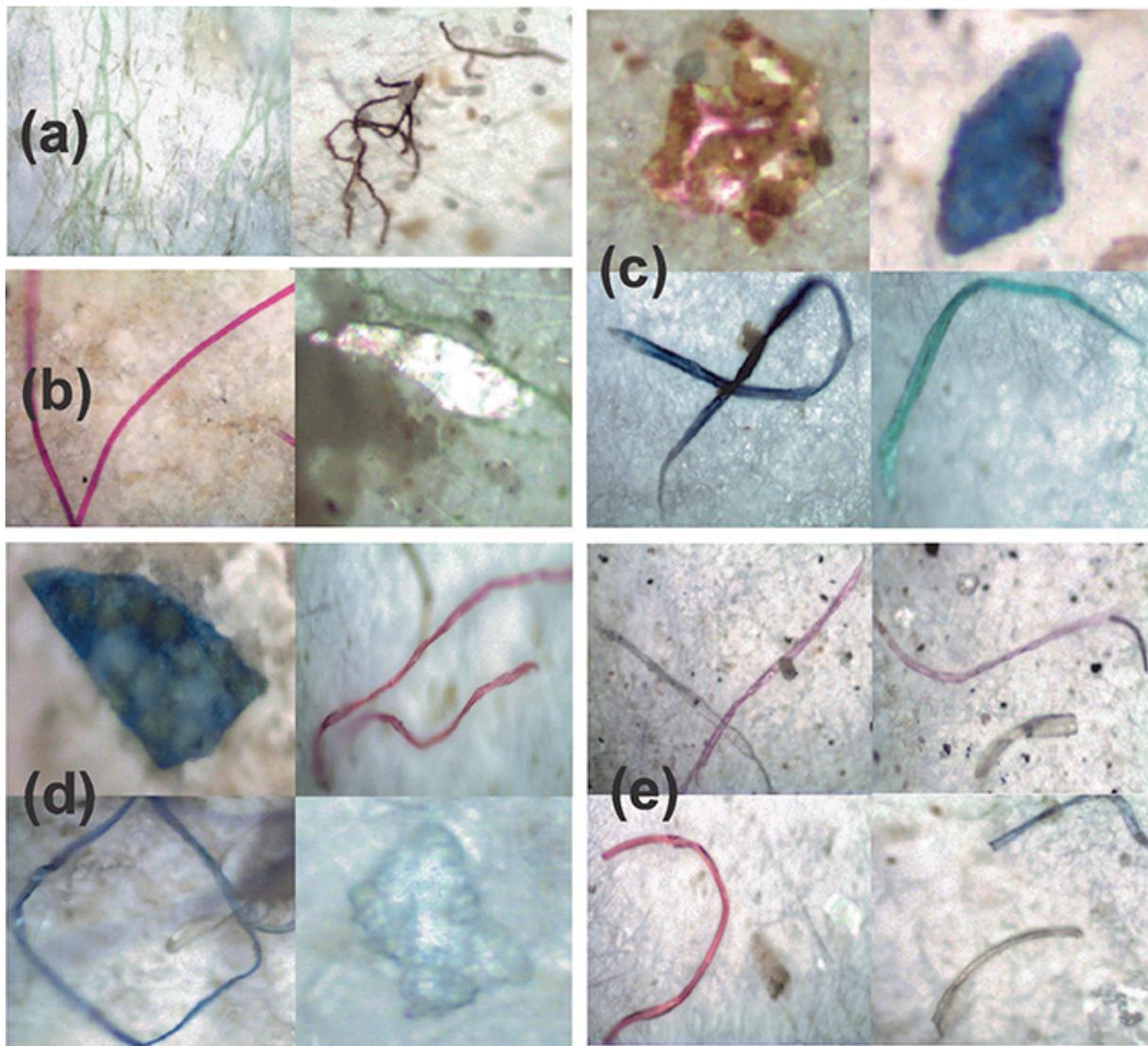


Figure 2

Non-microplastics and microplastics extracted in marine environment of Southern Vietnam: non-microplastic fibers (a); microplastics in Saigon urban canals (b), Can Gio (c), Ganh Rai (d), White Tiger oilfield (e)

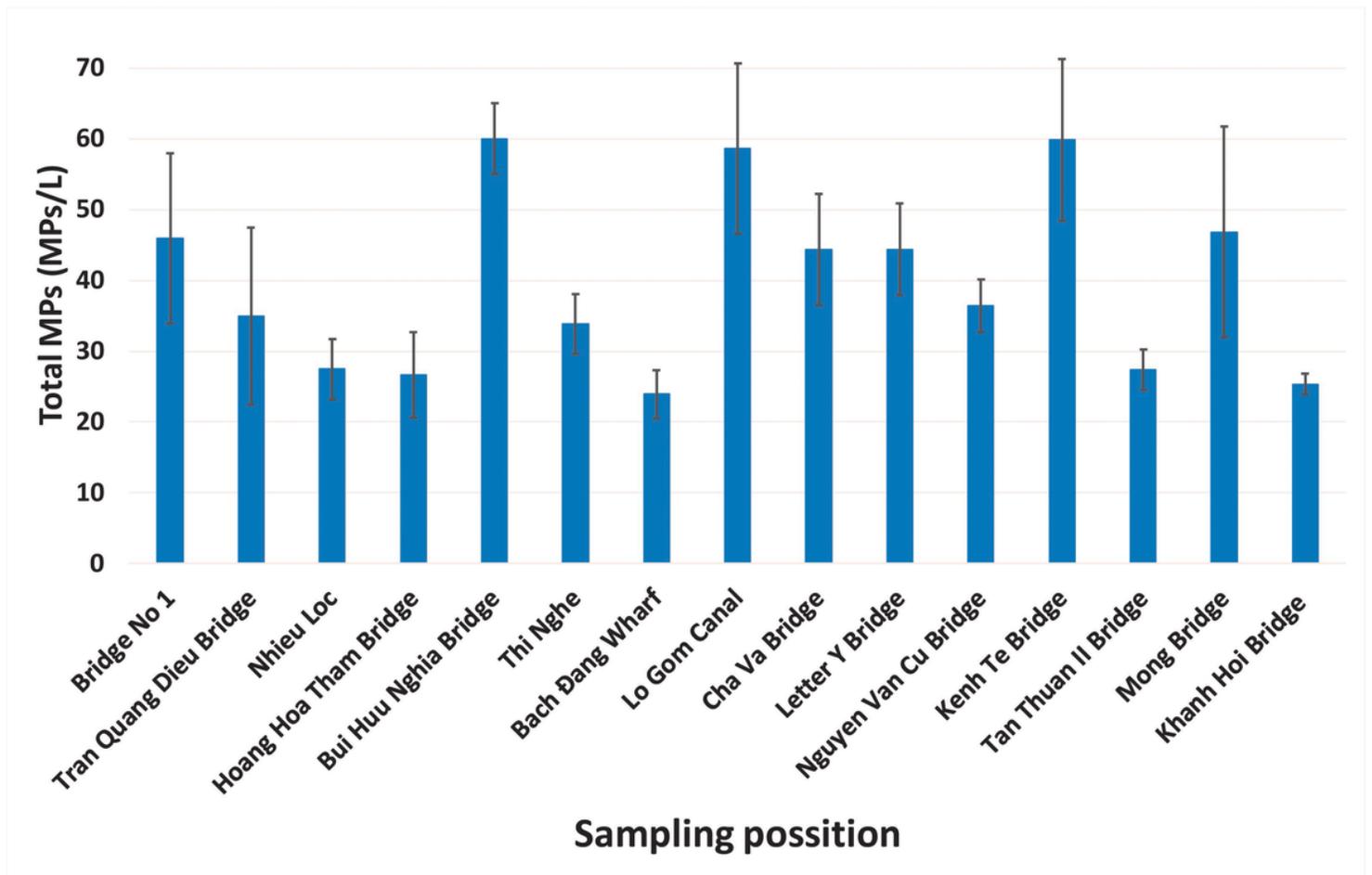


Figure 3

Total microplastics in Saigon urban canal systems *NL-TN Canal System* is from Bridge No.1 to Bach Ðang Wharf;
Tau Hu (TH) Canal System is from Lo Gom to Letter Y Bridge;
Te Canal (KT) is from Nguyen Van Cu Bridge to Tan Thuan Bridge;
Ben Nghe Canal (BN) is from Nguyen Van Cu Bridge to Khanh Hoi Bridge

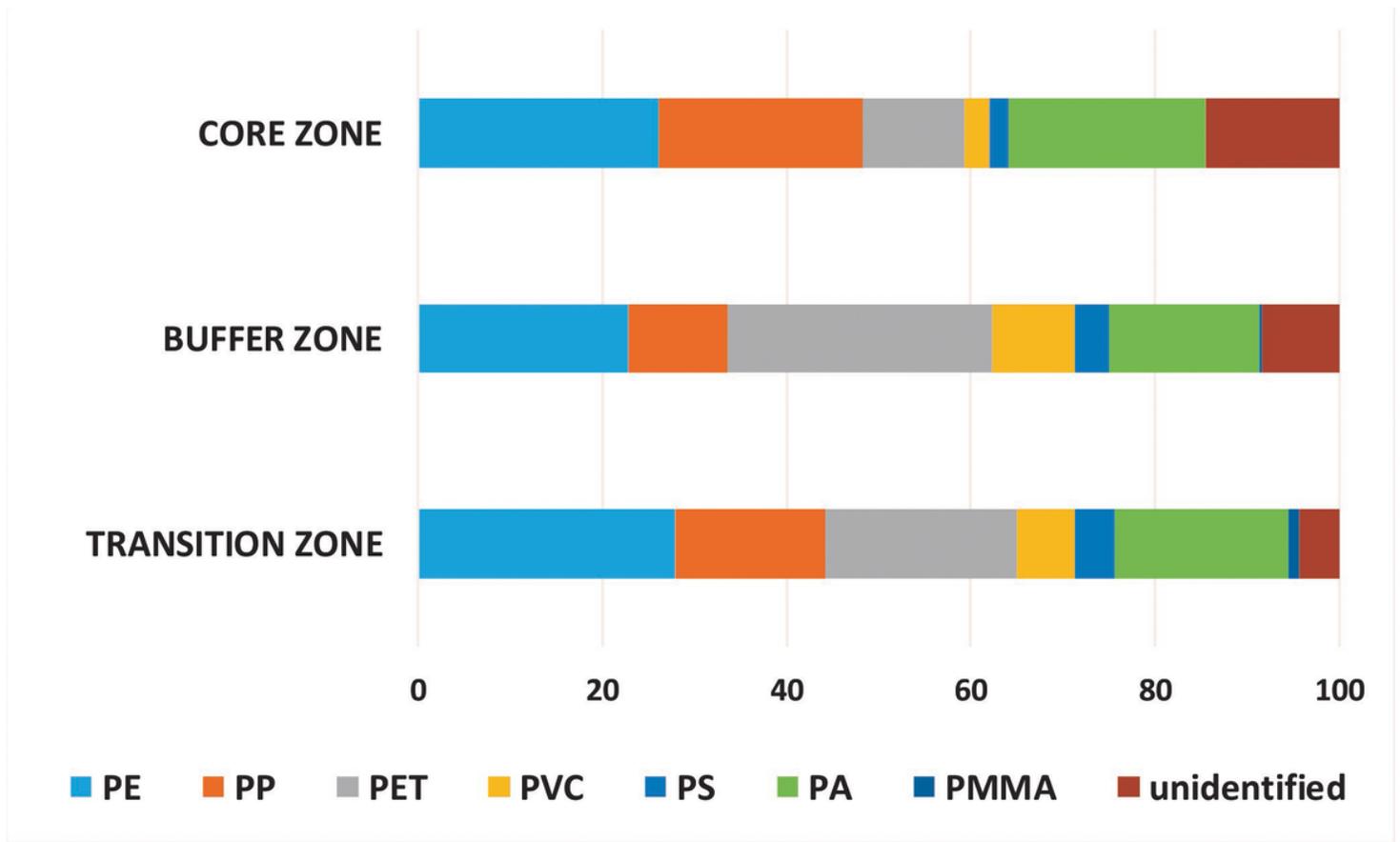


Figure 4

Microplastic distribution (%) in the zones of UNESCO Can Gio Biosphere Reserve

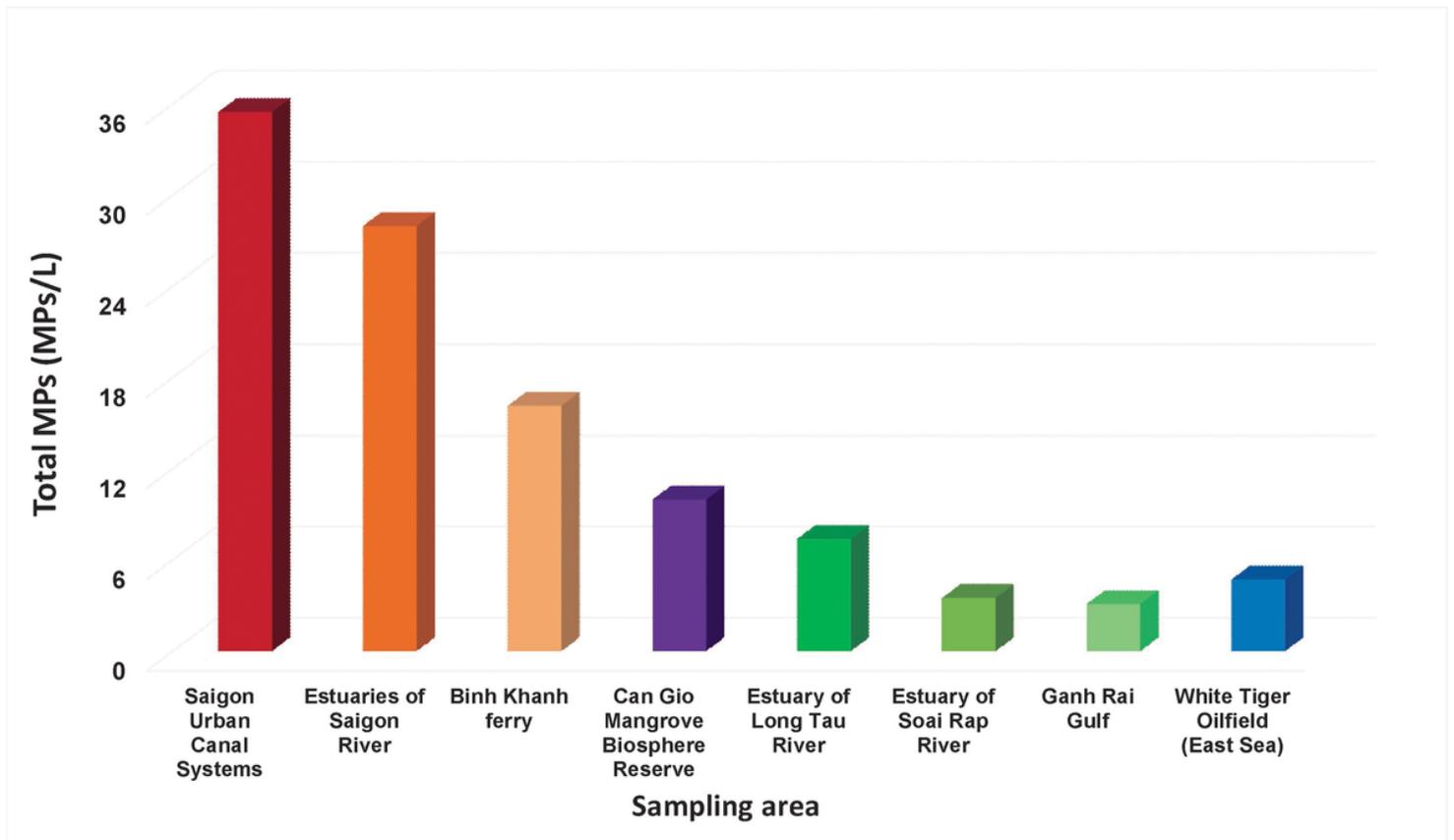


Figure 5

The decrease in microplastic concentration from Saigon to the East Sea through UNESCO Can Gio Mangrove Biosphere Reserve Binh Khanh ferry is the intersection of HCMC and Can Gio;

The confluence of Soai Rap River is the natural border of Can Gio with Mekong Delta;

Ganh Rai Gulf is the confluence of Long Tau River;

White Tiger Oilfield is in the East Sea belonging to Đong Nai city (not HCMC)

Supplementary Files

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- [KHUYENVOSupplementaryData04.03.2022.docx](#)