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Preliminary assessment of microplastic pollution in commercial freshwater fish species collected from four districts in Bac Ninh province

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Abstract. Microplastics (MPs) are of emerging widespread concern, but amount of research done in freshwater environments and organisms is scarce compared to that in marine environments. Thus, the MPs identification in four freshwater fish at four districts of Bac Ninh province were documented, including: common carp (*Cyprynus carpio*), grass carp (*Ctenopharyngodon idella*), tilapia (*Oreochromis niloticus*), and red-tilapia (*Oreochromis sp.*). MPs were found in the gastrointestinal tracts (GIT) of 100 % of examined fish. MPs abundances have significant differences between all freshwater fish collected from ponds and local markets (except for red-tilapia), while no difference by individual of each species neither in pond nor in local markets. The median sizes of microplastics ranged from 1410 µm to 2706 µm. The MPs were dominated by purple in color (in pond with average 38 %; in local market with average 33 %). µ-FTIR analysis showed that polymers found in fish GIT mainly were polyethylene terephthalate, polyethylene, polypropylene and nylon. These results showed that the microplastics was widely ingested by freshwater fish and help to aware people which fish are more contaminated with MPs to human consumption in Bac Ninh province.

Keywords: microplastics, freshwater fish, pond, local market, Bac Ninh.

Classification numbers: 3.2.3, 3.3.1, 3.6.1, 3.6.2.

1. INTRODUCTION

The continuing and exponentially growing usage of plastics worldwide has induced the global concern of their environmental impacts on biota [1]. Due to their special composition, plastics degraded by microorganisms very slowly and accumulate in marine and freshwater

ecosystems globally [2]. At present, environmental problems due to microplastics (MPs) contamination have become a global concern due to its significant impact on the ecosystem, food security and human health. When plastics reduce in size and exist as microplastics (< 5 mm), aquatic living organisms can ingest MPs depending on their feeding ecology [3]. The MPs ingestion by fishes is widespread and the most common sizes of MPs found in fish are less than 1 mm [4, 5]. Filtration is the main pathway for MPs ingestion of filter-feeding species [6], while predator species can incorporate MPs through their food transfer. Recently, many studies reported MPs ingestion in marine and freshwater fish species [5, 7 - 11]. Those authors reported that MPs seem to have the similar size as sediments and/or planktonic organisms so they can be bioavailable for a wide range of aquatic organisms. Moreover, due to the extensive usage of plastic facilities such as nets, ropes, pipes, etc. in fishing and aquaculture, aquatic organisms may be experienced more serious MPs ingestion [12 - 14]. Consequently, MPs may directly or indirectly affect the life quality of aquatic organisms such as accumulated in the gastrointestinal track and gills, thus dispersing throughout the body as well as to other tissues like liver and heart [15, 16]. From standpoints of food security and fisheries development, there is a need to conduct the investigation on the accumulation of MPs and its effects in commercial fish.

Bac Ninh province has many industrial zones and craft villages, resulting in environmental pollution. The amount of solid waste rapidly increases and has not been sorted at the source. According to the Department of Natural Resources and Environment in Bac Ninh province, there are about 500 tons of municipal, 200 tons of industrial, and two tons of medical waste daily generated. In addition, the amount of domestic solid waste has increased by 10 %, industrial solid waste by 15 %, and medical waste by 8 % each year [17]. Among the types of solid waste, plastic waste accounts for a large amount of solid waste in local landfills (8 - 12 %). Up to 90 % of plastic waste go to landfill and incineration, and only the remaining 10 % has been recycled [18]. To date, there has been no study on microplastic contamination in commercial freshwater fish species in Bac Ninh province. Thus, this study aims (1) to assess the abundance of MPs, colors, shapes and sizes, and (2) to identify the polymer types in different commercial freshwater fish species in Bac Ninh province, including common carp (*Cyprynus carpio*), grass carp (*Ctenopharyngodon idella*), tilapia (*Oreochromis niloticus*), and red-tilapia (*Oreochromis sp*.).

2. MATERIALS AND METHODS

2.1. Study sites

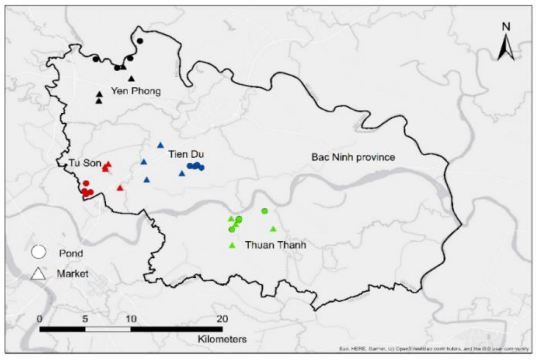

Figure 1. Study sites to collect fish species from ponds and local markets of four studied districts in Bac Ninh province.

To study the microplastic pollution in Bac Ninh province, commercial fish samples were collected in 4 districts (Yen Phong, Tu Sơn, Tien Du and Thuan Thanh) (Figure 1).

2.2. Sample collection

All equipment for sampling were cleaned to avoid any contaminants that could affect the result of the study. Fish species were collected from aquaculture farming (ponds) and local markets. The separated intestine was removed all fat around the intestines and then washed with distilled water before digesting to analyze microplastics. The number and average weight of fish were presented in Table 1.

Table 1: Number of fish species collected from ponds and local markets in Bac Ninh province.

	Fish species	Yen Phong	Tu Son	Tiên Du	Thuan Thanh
Fish collected from ponds	Tilapia	3 (450)	5 (503)	3 (425)	3 (535)
	Common carp	4 (800)	4(500)	4 (650)	6 (620)
	Grass carp	2 (600)	5 (800)		4 (770)
	Red-tilapia				2 (350)
Fish collected from local markets	Tilapia	3 (1065)	3 (1100)	3 (1000)	5 (1650)
	Common carp	4 (1680)	4 (2500)	3 (1430)	3 (2250)
	Grass carp	3 (2300)	5 (2950)	4 (1660)	4 (3560)
	Red-tilapia		5 (1650)	5 (950)	

Note: Data in the bracket presents the average weight (gram) of each fish species.

2.3 Sample processing and microplastic analysis

Table 2. Total microplastic particles extracted from intestine of fish species for µFTIR analysis.

	Districts	Tilapia	Common carp	Grass carp	Red-tilapia
Pond	Yen Phong	15	9	10	-
	Tu Son	20	11	6	-
	Tien Du	11	2	-	-
	Thuan Thanh	13	17	7	23
Local market	Yen Phong	31	11	21	-
	Tu Son	34	19	16	22
	Tien Du	45	33	23	11
	Thuan Thanh	51	14	18	-
	Total	**220**	**116**	**101**	**56**

Before isolating MPs from the gastrointestinal tract (GIT) of fish, the GIT was thoroughly washed with saturated NaCl to remove fat tissue. Subsequently, to separate MPs particles sticking to the GIT, KOH: NaClO solution (30 mL 50 %) was added and then digested at 50 °C for 48 hours until digestion completely [19]. Based on the GESAMP recommendation when visual observation is performed without systematic analysis of the polymers, fiber measurement was set up with the minimum length at 300 µm and the maximum length at 5000 µm, and for fragment measurement with a minimum area size of 45,000 μm^2 and a maximum size at 25,000,000 μm^2 [20]. All fractions > 250 µm was transferred into a clean 250 mL glass beaker using filtered NaCl saturated solution (with density 1.18 g mL^{-1}). This step was repeated at least

5 times to ensure the retrieval of all microplastic items. The overflowed solution was filtered on a cellulose nitrate membrane (0.45 μm pore size and 47 mm diameter) using a glassware filtration unit. Finally, the membrane filters were observed under the stereomicroscope Leica S9i equipped. After being counted by categories, a total of 56 - 220 MP particles of each fish species was randomly selected for identification of the polymeric structure through the micro-Fourier Transform Infrared Spectroscopy (μFTIR) instrument (Nicolet In10Mx Dual Director microscope, Thermo Fisher Scientific) according to Faure *et al*. [21]. Results are expressed in % polymer type of particles.

2.4. Quality control

The quality control was set up based on the recommendations of previous study [22]. All tools and glassware were washed three times with filtered distilled water, and operators were fully equipped with cotton lab clothes and nitrile gloves. The NaCl solution (density 1.18 g mL^{-1}) was filtered before use on cellulose nitrate membrane (porosity 0.45 μm) and kept in glass bottles. Distilled water was used as a blank during the entire treatment process. No microplastic were detected in the blank samples of any procedure, which indicated that the microplastic contamination during the experiment was negligible.

2.5. Statistical analysis

Statistical analyses were performed with Excel 2016 and SPSS 16.0 statistical software. Data are indicated as mean ± standard error (SE). Data were analyzed by One-way ANOVA followed by a T-Test's test for mean comparison of the significant differences between sampling sites and/or fish species, with a significant difference at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Abundance microplastics in fish

Table 3. The abundance of microplastic in gastrointestinal tract of fish species collected from ponds and local markets of districts in Bac Ninh province (items/g ww GIT).

	Districts	Tilapia	Common carp	Grass carp	Red-tilapia
Pond	Yen Phong	$2.6 \pm 0.4^{a,1}$	$3.3 \pm 0.9^{a,1}$	$4.0 \pm 0.6^{a,1}$	-
	Tu Son	$3.2 \pm 1.2^{a,1}$	$3.7 \pm 0.5^{a,1}$	$5.1 \pm 1.0^{ab,1}$	-
	Tien Du	$2.5 \pm 1.1^{a,1}$	$4.7 \pm 0.1^{ac,1}$	-	-
	Thuan Thanh	$2.9 \pm 0.2^{a,1}$	$2.1 \pm 1.2^{a,1}$	$3.3 \pm 0.1^{a,1}$	$2.9 \pm 0.1^{a,1}$
Local market	Yen Phong	$10.5 \pm 06^{b,1}$	$6.4 \pm 0.8^{b,2}$	$5.2 \pm 0.9^{ab,2}$	-
	Tu Son	$18.1 \pm 0.4^{b,1}$	$6.3 \pm 0.7^{b,2}$	$7.0 \pm 1.0^{b,2}$	$2.0 \pm 0.8^{a,2}$
	Tien Du	$11.1 \pm 1.1^{b,1}$	$5.4 \pm 1.0^{bc,23}$	$7.4 \pm 1.3^{b,2}$	$1.5 \pm 0.04^{a,3}$
	Thuan Thanh	$10.7 \pm 0.1^{b,1}$	$5.6 \pm 0.4^{bc,2}$	$6.7 \pm 0.3^{b,2}$	-

Note: Different letters denoted the significant differences between fish collected from ponds and local markets ($p < 0.05$). Different numbers denoted the significant differences between fish species ($p < 0.05$).

MPs were recorded in all fish species of 4 districts (Table 3), in varying abundance. Across all districts, means abundance of microplastics in GIT of fish varied for either each fish species or each site. Previous studies showed that the presence of MPs was verified in the 100 % of

commercial freshwater fishes [9, 10, 23]. In this study, the abundances of MPs in the intestine of fish had strong relationship increased with the size of fish (r^2 = 0.6691 - 0.8297), being higher in the big size fish (1000 - 3560 grams) collected from the local markets and lower in the small size fish (350 - 800 grams) collected from the ponds, except for red-tilapia species (Figure 2). Many studies indicated that MPs abundances have a positive linear relationship with fish body size [10, 24, 25] because large fish require more food to maintain their higher energy needs, resulting in accidental ingestion more MPs by fish for food instead of their prey [24]. In fish species collected from local markets, MPs abundances were highest values in tilapia species, within the range of 10.5 - 18.1 items/g ww of GIT, following by grass carp (5.2 - 7.4 items/g ww of GIT) and common carp (5.4 - 6.4 items/g ww of GIT). According to the previous studies, MPs ingested by fish depends not only on age, increase in body size, but also on feeding habit, habitat preferences or trophic position [26 - 29]. Therefore, tilapia is a filter-feeding omnivorous and inhabiting shallow species showed relatively higher abundance MPs than other freshwater species in this study [7 - 9, 30]. In the meanwhile, MPs abundance of fishes collected from ponds of districts was no significant difference by fish species ($p > 0.05$). This can explain that young fish with lower energy needs of any fish species consume less food, leading to the less direct or indirect ingestion of MPs. These findings suggest that fish body size or age may help explain MPs abundances in fish species.

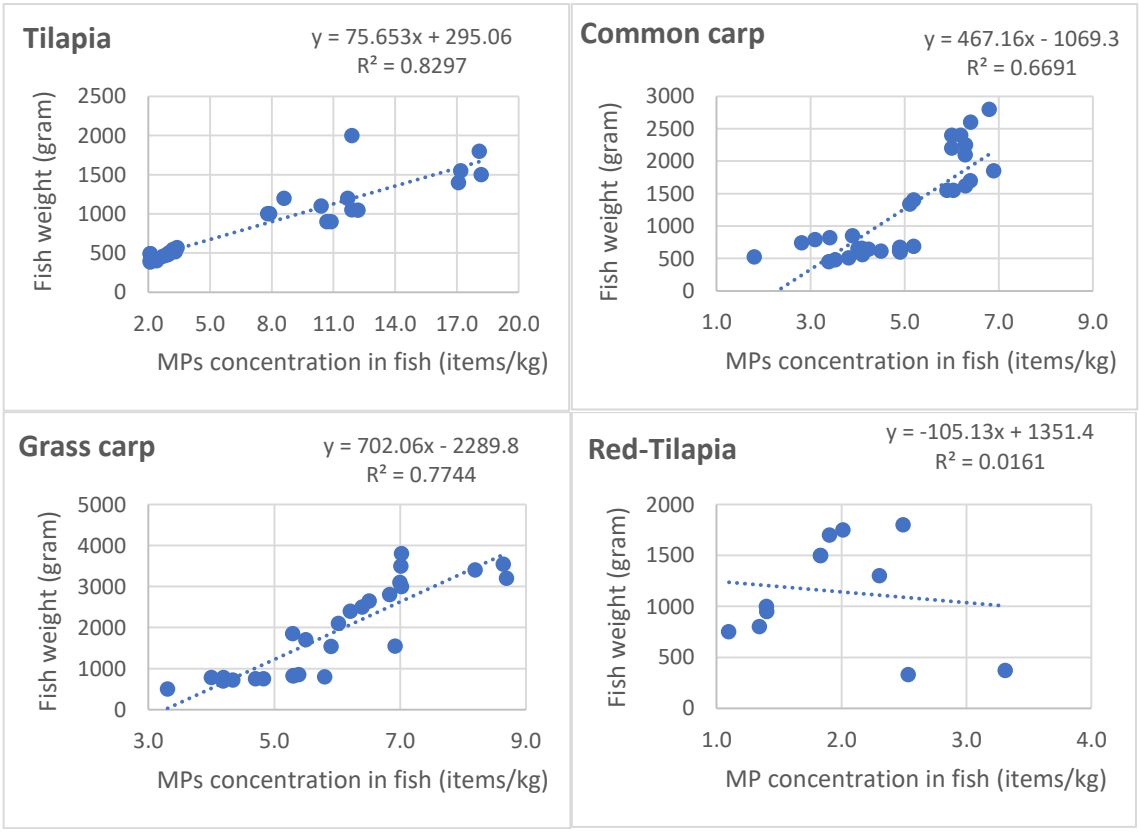

Figure 2. Relationship between MPs concentrations and fish sizes.

3.2. Microplastic size and color detected in fish tissue

In terms of the color, shape and size of MPs, colors identified include pink, blue, white (transparent), purple, yellow, green and orange (Figure 3); shapes such as plastic fragment, fiber, platic films were detected in GIT of fish. In all studied fish species collected from districts in Bac Ninh province, the dominant color was purple (average 33 % for fish in local market and 38 % for fish in pond) and dominant shape was fiber (87 %). Kasamesiri and Thaimuangphol [31] found that dominant color was blue (56.9 %) and shape was fiber (86.9 %) in freshwater fish *Puntiolites proctozysron*. Black dominated (45 – 48 %) in the freshwater fish *Gambusia affinis* of all sites [24]. In this study, the color analysis indicated that the dark colors such as purple or blue of MPs was significantly more common than light color in freshwater fish. This can be opposite from the observations of Wang *et al*. [8] and Sun *et al*. [9] that the light colors (white and yellow) of MPs was significant common than dark color ones in freshwater fish. It can be due to the feeding habitats of fish species (omnivore, carnivore and herbivore). Three out of four fish species in this study are omnivores consuming a variety of aquatic insects, copepods, rotifers, zoobenthos and protozoa in the diet and dark colors of MPs are thought to me more similar to that of the food [32].

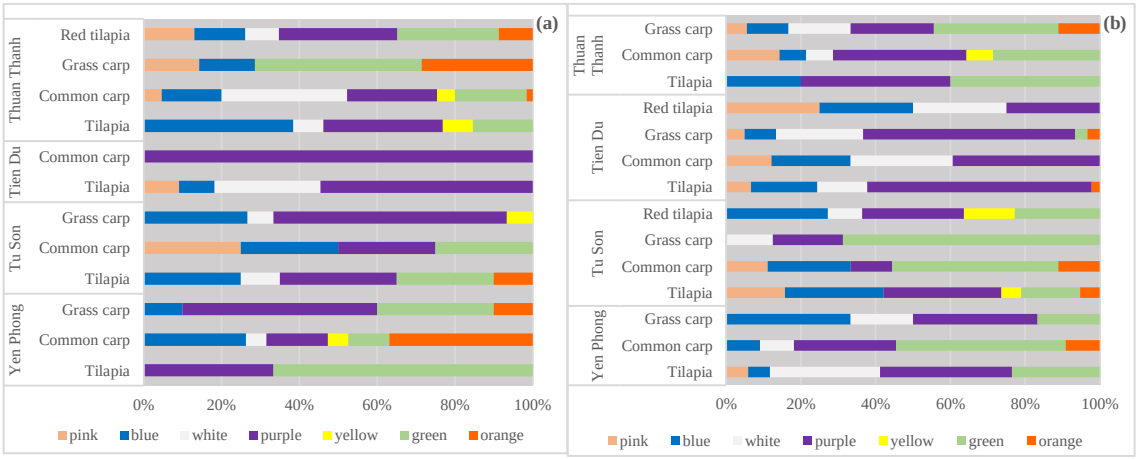

Figure 3. Color distribution of microplastics in fish species collected from ponds (a) and local markets (b).

Table 4. Median size (µm) of microplastics detected in digestion system of common fish species.

	Districts	Tilapia	Common carp	Grass carp	Red-tilapia
Pond	Yen Phong	2191.02	2232.18	1513.79	
	Tu Son	1906.21	1752.21	2014.58	
	Tien Du	2259.05	2613.29		
	Thuan Thanh	1731.04	2653.81	2706.31	1999.11
Local market	Yen Phong	2297.35	1499.42	2545.64	
	Tu Son	1410.78	1598.15	1963.73	2059.89
	Tien Du	2551.91	2217.29	2189.64	2159.44
	Thuan Thanh	1093.95	1532.75	2623.79	

From the observation of the MPs's size, the median length ranged from 1410 µm to 2706 µm (Table 4). At present, there is no clear definition of the size classification of microplastics.

Most of the microplastic observed in the fish samples were small, with size less than 2 mm accounting for more than 50 % of the total in all fish samples (Figure 4). However, Wang *et al*. [8] reported that the proportion of MPs < 0.5 mm in size in omnivorous and herbivorous were 59 % and 52 %, respectively. Similarly, high proportion of MPs < 1.0 mm in size was observed in GITs of fish, accounted for 85% in *Oreochromis niloticus* and 69.2 % in *Cirrihinus molitorella* [9]. Under the cumulative analysis for size classification in this study, the proportion of fish samples containing microplastics < 3 mm in size ranged from 50 % to 89 % of all fish samples. In fact, it is very difficult to compare the sizes of MPs in different fish that live in different habitats and consume different feeds.

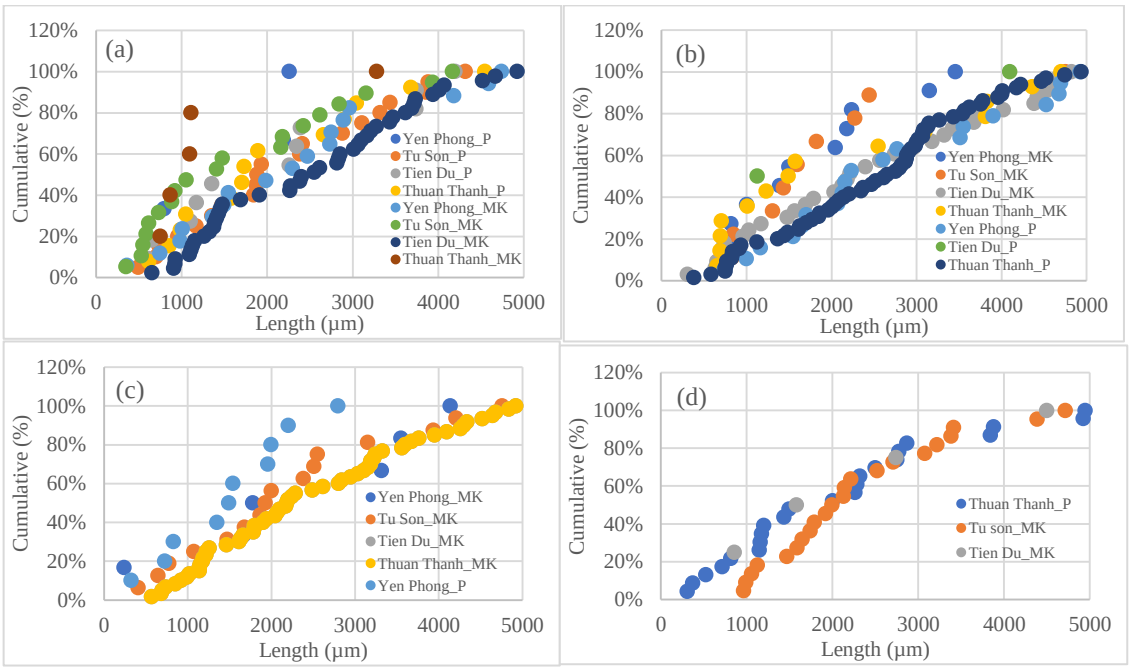

Figure 4. Observed cumulative size distribution of microplastics in intestine of fish species collected from ponds and local market of districts in Bac Ninh province: (a) tilapia; (b) common carp; (c) grass carp; (d) red-tilapia.

3.3. Polymer composition

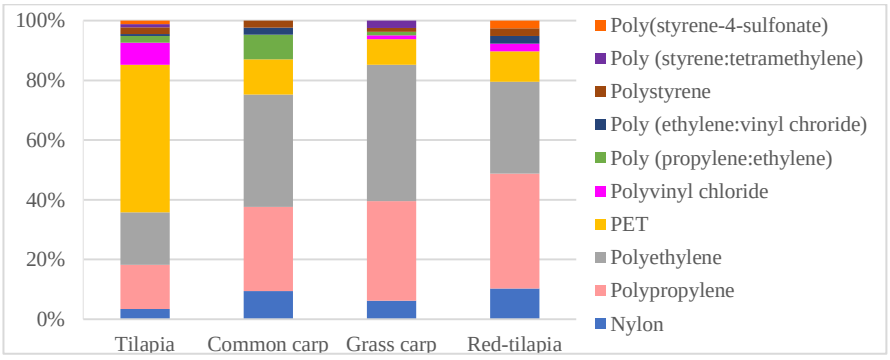

Figure 5. Component of polymer types in GIT of different freshwater fish.

The results of µFTIR showed that polypropylene (PP: 15 - 38 %), polyethylene (PE: 18 - 46 %), polyethylene terephthalate (PET: 9 - 49 %) and nylon (3 - 10 %) were the major polymers in the gastrointestinal tracts of fish species collected from ponds and local markets in Bac Ninh province (Figure 5). However, each fish species in this study had a dominant polymer type. For instance, PET was observed as a dominant polymer type in tilapia, accounted for 49 % in GIT. Polymers of PET and nylon, which are denser than water, are more likely to sink in the sediment and fishes that live and feed on or near the bottom of water column of the aquatic ecosystems (e.g. tilapia and common carp) may ingest those polymers accidentally. Meanwhile, predominance of PE and PP was observed in common carp, grass carp and red-tilapia. It is well known that PE and PP have low density (less than 1.0 g cm^{-3}) and float in the surface water which makes it more available for benthicpelagic and pelagic fishes such as common carp, grass carp and red-tilapia [31].

4. CONCLUSIONS

This work is the first report of the occurrence of MPs in commercial freshwater fish species of ponds and local markets of districts in Bac Ninh province. MPs were detected in all fish samples and big fish contained higher abundance in GIT than that in small fish. Fiber was the main type of MPs and purple was the most common color. Most of MPs in the fish sample were < 3 mm in size. The MPs found in GIT of fish were mainly PET, PE, PP and nylon. However, the results indicated that the polymer types of MPs in different fish species showed certain differences and specificities. The results of this study can be used as reference for future research of microplastic contamination in other commercial fishes in Viet Nam.

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CRediT authorship contribution statement. This manuscript was written through the contributions of all authors. Huong Mai: writing the manuscript, methodology, data collection and statistical analysis. Van Hoi Bui, Thanh Duong Dao: fish sampling at study sites. Thi Thao Nhi Ngo, Nguyen Danh Thien: conducting the extraction and digestion to get MPs from fish and lecturing microplastics using Leica stereomicroscope, and data statistical analysis. Thi Thom Dang, Van Manh Do: µFTIR analysis of microplastics.

Declaration of competing interest. The authors declare no competing financial interests.

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