



Research article

Microplastics in surface water and tissue of white leg shrimp, *Litopenaeus vannamei*, in a cultured pond in Nakhon Pathom Province, Central Thailand

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Abstract: The presence of microplastics in commercially important seafood species is a new issue of food safety concern. Although plastic debris has been found in the gastrointestinal tracts of several species, the prevalence of microplastics in edible shrimp tissues in Thailand has not yet been established. For the first time, the gastrointestinal tract (GT), hepatopancreas (HEP), muscle (MU) and exoskeleton (EX) of farmed white leg shrimp (*Litopenaeus vannamei*) from commercial aquaculture facilities in Nakhon Pathom Province, Thailand, were analyzed for microplastics (MPs). The number of MP items per tissue was 27.36 ± 2.28 in the GT, 17.42 ± 0.90 in the HEP, 11.37 ± 0.60 in the MU and 10.04 ± 0.52 in the EX. MP concentrations were 137.78 ± 16.48 , 16.31 ± 1.87 , 1.69 ± 0.13 and 4.37 ± 0.27 items/gram (ww) in the GT, HEP, MU and EX, respectively. Microplastics ranged in size from <100 to 200–250 μm , with fragment-shape (62.07%), fibers (37.31%) and blue (43.69%) was the most common. The most frequently found polymers in shrimp tissue organs and pond water were polyethylene terephthalate (PET), polyvinyl acetate (PVAc) and cellulose acetate butyrate (CAB). Shrimp consumption (excluding GT and EX) was calculated as 28.79 items/shrimp/person/day using Thailand's consumption of shrimp, MP abundance and shrimp consumption. The results of the study can be used as background data for future biomonitoring of microplastics in shrimp species that are significant from an ecological and commercial perspective. MP abundance in farmed *L. vannamei* may be related to feeding habits and the source of MPs could come from the aquaculture facilities operations.

Keywords: microplastics; FT-IR; surface water; tissue of white leg shrimp

1. Introduction

Globally there has been a rapid increase in plastic production which resulted in a large amount of plastic waste released into the environment. Plastic waste causes problems to the environment due to its low recycling rate and insufficient waste management. Since the outbreak of the COVID-19 epidemic, Beuson et al. [1] estimated that daily 1.6 million tons of plastic wastes have been discarded worldwide. Plastic particles less than 5 mm are referred to as microplastics (MPs). MPs originate from the direct use of materials like textile fibers or the natural breakdown of polymers. MPs, which are frequently found in nature, threaten aquatic ecosystems and living resources when contaminants flow through aquatic habitat and the species in that habitat ingest MPs. Over the past ten years, an increase in research on environmental problems caused by microplastics has been conducted globally [2–8]. Prior studies sought to investigate microplastic pollution in regional or national waters, as well as microplastic accumulation in living organisms.

Water environments are threatened by the mixing of microplastics from various sources, which could also endanger people who consume contaminated fish, shrimp and seafood [9]. Potential effects of MPs on aquatic species are caused by the physical and chemical reactions of these ingested plastics [10–11]. MPs can have negative effects because of their own particles, materials added during the production of plastics and contaminants absorbing to plastic debris in the environment [11]. According to research on the toxicity of MPs, these substances have a physical and chemical impact on aquatic organisms. Among these effects are genotoxicity, oxidative stress, behavioral disorders, reproductive problems, mortality and a decrease in the rate of population growth [4,10–11]. Aquatic species can become polluted by microplastics in the water, contaminated food sources or other living organisms [12–14].

Microplastic accumulation in important shrimp species could be harmful to human health [4,11]. Nowadays, it is known that plastic waste affects more than 660 marine species [15,16]. In the marine biota, ingested MPs usually damage or cause fish a false feeling of fullness before being ejected through feces or occasionally remaining in the gastrointestinal tract. In other instances, it is fragmented into smaller pieces and passes past the intestinal wall before reaching the circulatory system [6,11,17]. Growing evidence suggests that microplastics can enter the food chain. MPs can be found at a variety of trophic levels, including plankton, bivalves and fish that humans consume [18,19]. This situation raises concerns regarding the detrimental impacts of bioaccumulation from one trophic level to another.

Litopenaeus vannamei, commonly known as the white leg shrimp, is a significant aquatic organism that can provide people with a high-quality source of protein. Shrimps are generally vulnerable to microplastic pollution due to their scavenging mode of feeding and multipart intestine [20]. MPs were found in the intestines of 30.9% of *Fenneropenaeus indicus* [20] and 63% of brown shrimp, *Metapenaeus monoceros* [21]. The average MPs items per gram of intestine in the penaeid shrimps *Penaeus monodon* and *M. monoceros* were 3.40 ± 1.23 and 3.87 ± 1.05 , respectively [22]. It has been reported that the accumulation of fragmented MPs in the digestive tract of grass shrimp (*Palaemonetes pugio*) is greater than that of spheres and fibers [23]. In industrialized shrimp farming, plastic materials such as polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), polytetrafluoroethylene (PTFE) and polypropylene (PP) are utilized to construct the majority of culture ponds and other equipment. As a result, the impact of MPs on shrimp cannot be neglected. Although MP contamination in aquatic environments is a rising worldwide issue, there is little information in the literature about the uptake by commercial freshwater shrimp.

Aquaculture has a significant impact on the economy of Thailand and the rest of the world. The

productivity and quality of aquaculture products may be impacted by the water quality of the pond [24]. Microplastic accumulation in the hydrological system could be ingested by species at low to high trophic levels [25]. Therefore, research on microplastic pollution in aquaculture ponds is crucial for environmental management and the advancement of sustainable aquaculture. The presence and composition of microplastics in several tissues including the gastrointestinal tract (GT), hepatopancreas (HEP), muscle (MU) and exoskeleton (EX) of white leg shrimp (*Litopenaeus vannamei*) were examined in light of this information. In addition, microplastics at the surface water level of a cultured shrimp pond were also investigated.

2. Materials and methods

2.1. Research area and sample collections

Samples for analyzing microplastics were obtained from white leg shrimp (*Litopenaeus vannamei*) and water from the surface level of ponds. Fresh white leg shrimp samples were collected in November 2021 from seven local shrimp agriculture ponds in Central Thailand (Figure 1) using a specially designed seine net. These areas represented various cohorts or populations of white leg shrimp. Surface water and shrimp samples were collected once from each pond.

At each pond, five 1-liter plastic bottles were used to collect surface water samples (depth 0–5 cm). To reduce disturbance from re-suspended sediments, water samples were taken gently.

A total of 105 white leg shrimp individuals (15 from each pond) were collected and kept frozen in an icebox before being transported to the Faculty of Liberal Arts and Science's Zoology Laboratory, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province, Thailand.

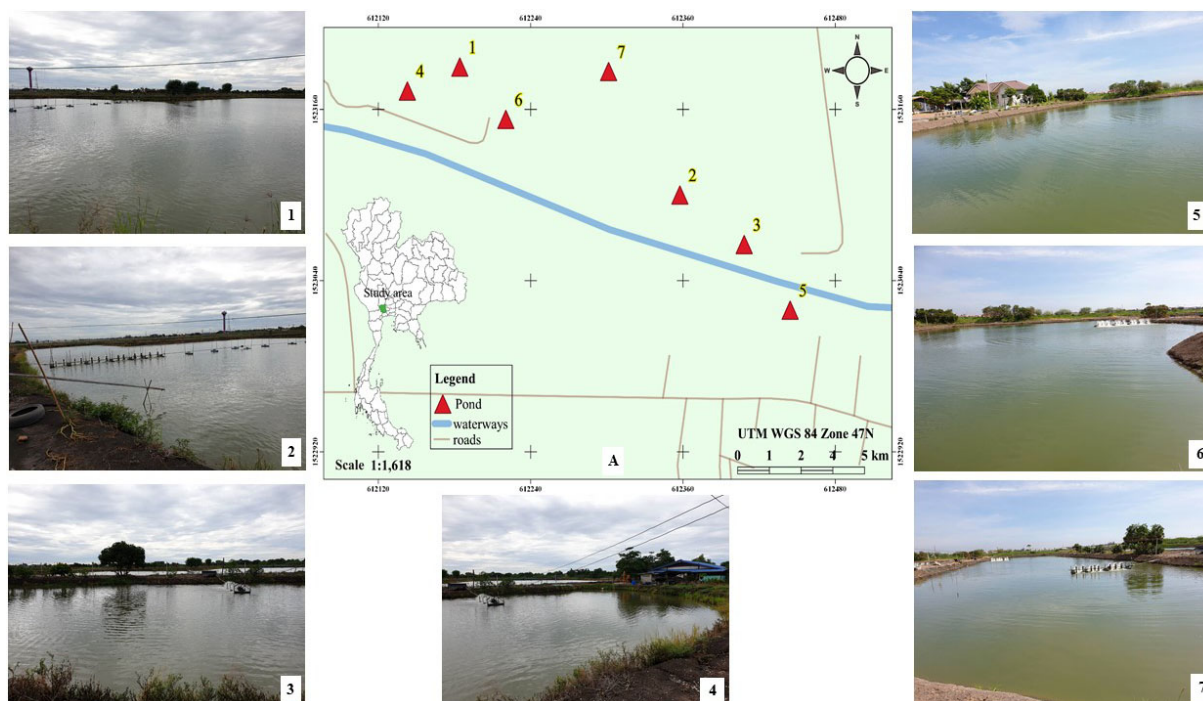


Figure 1. Seven collecting ponds (1 to 7) in the Nakhon Pathom Province, Thailand. A map showing seven ponds was drawn using the QGis 3.14.1 program (<https://www.qgis.org/en/site/>).

2.2. A sample of pond surface water analysis

MPs were detected in pond surface water samples using wet peroxide oxidation [26]. Each water sample was transferred to a 200 mL conical flask. The samples were digested with 20 mL of 30% hydrogen peroxide (H₂O₂) and left for 24 hours. Following that, the samples were filtered through a nylon membrane filter (Whatman, Kent, UK; pore size 0.45 µm; diameter, 47 mm), using a pressure filtration apparatus. Each membrane filter was then placed onto a clean Petri dish, wrapped in aluminum foil and dried for two days at 50 °C in a drying cabinet.

2.3. Shrimp tissue digestion

Before being dissected, the shrimp were defrosted at room temperature. Each shrimp's exoskeleton was rinsed twice with deionized water (DI) to eliminate any potential contamination from the plastic freezer bags in which the shrimp were stored. Each shrimp was weighed using a digital scale and its total length was determined using a ruler.

Metal forceps and metal scissors were used to individually dissect the specimens on metal trays and remove their gastrointestinal tracts (GI), hepatopancreas (HEP), muscle (MU) and exoskeleton (EX), which were then cleaned with DI after dissection (Figure 2). Each tissue organ was moved and weighed separately before being placed into a 100 mL conical flask.

Before starting the lab work, all lab surfaces and glassware were thoroughly cleansed with 70% ethanol and ultrapure water to make sure there was no MP contamination. Additionally, after removing each tissue organ from each specimen, the forceps were thoroughly cleaned to avoid MP cross-contamination between the specimens. Finally, to avoid airborne MP contamination, aluminum foil was immediately placed over the Petri dishes containing each tissue organ sample.

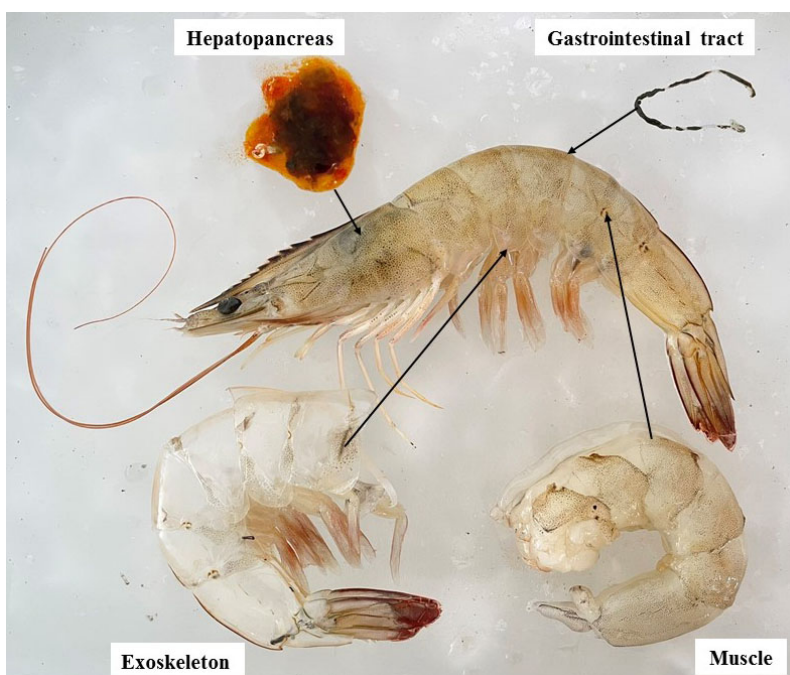


Figure 2. *Litopenaeus vannamei*'s body and its tissues.

MPs were extracted from each tissue organ of the specimens using a 30% H₂O₂ solution. For each conical flask, 20 mL of 30% H₂O₂ was added to breakdown the soft tissue, which was then heated at 60 °C in a shaken water bath at 150 rpm for three hours or until all of the organic matter was digested [27]. Parallel to soft tissue breakdown, the blanks were examined for the presence of MPs. No MP particles were detected in the blanks.

2.4. Potassium formate (HCO₂K) flotation and filtration

By using HCO₂K flotation and filtration, MPs were isolated from the dissolved organic matter solution [28]. Each sample was put into a glass separation funnel and saturated with HCO₂K (99%) until it reached a concentration of 1.6 g ml⁻¹. The samples were then kept at room temperature for a minimum of three hours. Undissolved organic residues and inorganic substances sank to the bottom of the glass containers while the less dense particles separated due to the saturated solution, resulting in a layer of MPs. The samples were subsequently filtered using a nylon membrane filter (Whatman, Kent, UK; pore size, 0.45 µm; diameter, 47 mm) and a pressure filtration apparatus. Each membrane filtered was then put onto a clean Petri dish, wrapped in aluminum foil and dried for two days at 50 °C in a drying cabinet.

2.5. Microplastic observation and polymer identification

Each filter was examined visually for the presence of MPs using a stereomicroscope (Leica EZ4E) and MPs were recognized based on their color and shape [29]. The MPs' morphologies were then divided into four categories: fiber, spherical, film (a thin layer) and fragment (a part of a larger plastic item) [30].

737 particles from the shrimp tissue organ and 304 particles from the surface pond water were manually evaluated using a Hyperion 2000 FT-IR microscope with a mercury-cadmium telluride detector (Bruker Daltonik, Billerica, MA, USA) at wavenumbers ranging from 4000 to 600 cm⁻¹, with 32 co-added scans and a spectral resolution of 4 cm⁻¹. The spectra that were gathered were compared to those in the Bruker database using OPUS software, version 7.5 (Bruker). Particles with a hit quality above 700 (maximum of 1000 hit quality) were accepted as verified polymers [31].

2.6. Data analysis

The number of plastics in each sample was counted and the mean number of plastic particles per sample was calculated considering all the samples analyzed. Each shrimp tissue organ, as well as the pond surface water, had the MP type, size and color examined and measured. To determine significant variations in MP abundance in shrimp tissue organs and pond surface water, one-way ANOVA and Tukey's (HSD) post hoc pairwise comparisons were carried out using SPSS software version 20.0. (IBM, Armonk, NY, USA). Additionally, Microsoft Excel 2013 (Microsoft Corp., Redmond, WA, USA) was used to generate graphs.

3. Result and discussion

3.1. Microplastic concentration in each tissue organ of shrimp

There was no MP contamination in the procedure blank samples. Within the study, 105 of *L.*

vannamei individuals (15 from each pond) were examined. The average length of the shrimps in Pond 1 was 11.39 ± 0.17 cm TL, Pond 2 was 10.37 ± 0.20 cm TL, Pond 3 was 11.47 ± 0.17 cm TL, Pond 4 was 15.12 ± 0.20 cm TL, Pond 5 was 17.12 ± 0.20 cm TL, Pond 6 was 18.32 ± 0.22 cm TL and Pond 7 was 25.12 ± 0.54 cm TL (Table 1).

In the 105 shrimp that were analyzed, 6949 plastic-like particles were confirmed as MPs. Of the particles, 41.33% were detected in the GT, 26.32% in the HEP, 17.18% in the MU and 15.17% in the EX.

Each individual shrimp that was investigated had MPs. The average MP item per an individual shrimp in the study was 66.17 ± 29.19 . When the four anatomical compartments were accounted for separately, the average MP item per tissue was 27.36 ± 2.28 in the GT, 17.42 ± 0.90 in the HEP, 11.37 ± 0.60 in the MU and 10.04 ± 0.52 in the EX.

MP concentrations were 137.78 ± 16.48 , 16.31 ± 1.87 , 1.69 ± 0.13 , and 4.37 ± 0.27 items/gram (ww) in the GT, HEP, MU and EX, respectively (Figure 3).

The MP content of the exoskeleton, hepatopancreas, muscle and gastrointestinal tract samples of *L. vannamei* from each pond is shown in Table 1.

Table 1. A summary of the total length of white leg shrimp in each pond, as well as the anatomical distribution of microplastics in shrimp.

Pond	Total length(cm)	Organ (n=15)	Organ wet weight (g/ww)	Total MPs (item)	Average MPs/organ
P1	11.39±0.17	Gastrointestinal tract (GT)	0.29±0.05	398	26.53±5.02 ^b
		Hepatopancreas (HEP)	1.13±0.25	307	20.47±3.85 ^b
		Muscle (MU)	4.35±0.24	115	7.67±0.93 ^a
		Exoskeleton (EX)	2.34±0.11	77	5.92±1.03 ^a
	F-value				9.950**
P2	10.37±0.20	Gastrointestinal tract (GT)	0.42±0.06	237	15.80±2.75 ^{ab}
		Hepatopancreas (HEP)	1.27±0.19	268	17.87±2.59 ^b
		Muscle (MU)	4.04±0.28	180	12.00±1.17 ^{ab}
		Exoskeleton (EX)	1.58±0.11	141	9.40±1.36 ^a
	F-value				3.279*
P3	11.47±0.17	Gastrointestinal tract (GT)	0.46±0.07	313	20.87±2.32 ^b
		Hepatopancreas (HEP)	0.10±0.16	185	12.33±1.18 ^a
		Muscle (MU)	4.17±0.50	111	7.79±1.14 ^a
		Exoskeleton (EX)	1.53±0.10	159	10.60±0.81 ^a
	F-value				14.135**
P4	15.12±0.20	Gastrointestinal tract (GT)	0.42±0.04	277	17.73±2.80 ^a
		Hepatopancreas (HEP)	2.57±0.23	266	17.73±2.80 ^a
		Muscle (MU)	7.23±0.44	219	14.60± 9.83 ^a
		Exoskeleton (EX)	2.29±0.08	143	11.00±1.14 ^a
	F-value				2.647
P5	17.12±0.20	Gastrointestinal tract (GT)	0.18±0.01	504	33.60±4.93 ^b
		Hepatopancreas (HEP)	1.36±0.15	293	19.53±1.07 ^a
		Muscle (MU)	11.02±0.19	176	11.73±1.32 ^a
		Exoskeleton (EX)	3.49±0.13	160	10.67±1.03 ^a
	F-value				15.817**
P6	18.32±0.22	Gastrointestinal tract (GT)	0.46±0.16	399	26.60±6.96 ^b
		Hepatopancreas (HEP)	2.55±0.10	232	15.47±1.61 ^{ab}
		Muscle (MU)	13.34±0.19	198	13.20±1.76 ^{ab}
		Exoskeleton (EX)	3.14±0.15	166	16.58±1.98 ^a
	F-value				3.410*
P7	25.12±0.54	Gastrointestinal tract (GT)	0.16±0.01	744	49.60±9.97 ^b
		Hepatopancreas (HEP)	2.88±0.18	278	18.53±2.01 ^a
		Muscle (MU)	15.54±0.30	195	13.00±1.85 ^a
		Exoskeleton (EX)	3.69±0.21	208	13.87±1.32 ^a
	F-value				11.150**

*The mean±S.E. difference is significant at the 0.05 level ($P<0.05$), **The mean±S.E. difference is significant at the 0.01 level ($P<0.01$): passed Tukey's post-hoc multiple comparisons test.

The number of MP items between tissues showed significant differences ($P < 0.05$), in which the $GT > HEP > MU > EX$ (Figure 3a), while the MPs per gram of tissue (ww) in the GT (137.78 ± 16.48) were significantly higher than those in the HEP (16.31 ± 1.87), in the MU (1.69 ± 0.13) and in the EX (4.37 ± 0.27) (Figure 3b). The abundance of MPs per shrimp in the four tissues was 40.04 ± 4.69 items/shrimp, while the abundance per gram of shrimp (ww) was 16.55 ± 1.08 items/g.

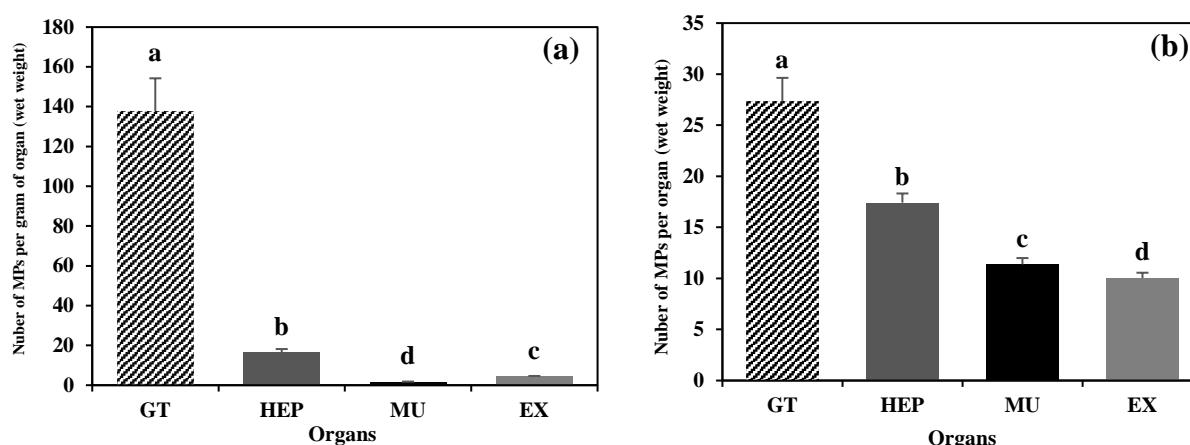


Figure 3. Mean (\pm S.E.; $n = 105$), (a) number of MPs per gram of organ represented as wet weight, (b) number of MPs per organ. Significant differences ($P < 0.01$) between organs are indicated by different letters.

The concentration of microplastic in seafood could increase as a result of an increase in marine microplastics [32]. Currently, several studies have been conducted to investigate the microplastic pollution of marine organisms. In the investigation of brown shrimp and tiger shrimp living in the Bay of Bengal, in Northern Bangladesh, Hossain et al. [6] found that the average microplastic values were $3.40 \pm 1.23/g$ and $3.87 \pm 1.05/g$. Microplastics (MPs) were in the forms of filament (57–58%) and fiber (32–57%) and were types of PA-6 and rayon polymer [33]. Gurjar et al. [34] found microplastics in the GT of three species of shrimp (*Metapenaeus monoceros*, *Parapeneopsis stylifera* and *Penaeus indicus*) on fishing grounds in the northern Arabian Sea with MPs/g values of 64.8 ± 24.6 , 78.5 ± 48.4 and 47.5 ± 38.0 , respectively. The average number of microplastics found in the gastrointestinal tract was 70.32 ± 34.67 MPs/g and six different types of plastic polymers were identified [34]. Wang et al. [35] observed 7.8 MPs/g in the GT and the GI of *Parapeneopsis hardwickii* from Hangzhou Bay, China. With the exception of those MPs that Curren et al. [36] observed in *Fenneropenaeus indicus*, the abundance of MPs in the GT of this study is higher than that of other penaeid shrimp studies (Table 2).

Reunura and Prommi [37] examined the levels of microplastic contamination in the gastrointestinal tracts of male freshwater shrimp and *L. vannamei* and found that they contained microplastics of 32.66 ± 5.10 , 32.14 ± 4.85 and 10.28 ± 1.19 MP/g. The various types of microplastics were identified as polyethylene, polycaprolactone, polyvinyl alcohol and acrylonitrile butadiene styrene [37]. Microplastic concentrations in the southern North Sea habitat region and channel area were examined by Devriese et al. [20] and found that 63% of shrimp samples had microplastics and it was determined that the average concentration was 1.23 ± 0.99 MPs/g [20]. However, the effect of microplastics on shrimp has been the subject of numerous research studies. Yoon et al. [38] investigated the number, size, color, type and presence of MPs in the heads and intestines of the marine organism *Litopenaeus vannamei*. Microplastics ranged in size from 1.73 to 3.8 MPs per 10 g of shrimp and the percentage of microplastics smaller than 100 μm was 77–92%, with blue showing the highest ratio.

Regarding the type of plastic, PET, PS, nylon and PVC were discovered, with PE and PP showing the highest ratio. Microplastics were identified in samples with heads and intestines to be 11.83 MPs/10 g and in those without heads and intestines to be 3.16 MPs/10 g [38].

In this study, four different types of MPs were present in all shrimp tissues: fibers, fragments, spheres and films (Figure 4). A total of 6949 MPs were extracted from shrimp. Of the MPs, 2872 were found in the gastrointestinal tract (GT), 1829 in the hepatopancreas (HEP), 1194 in the muscle (MU) and 1054 in the exoskeleton (EX). There were 6,949 microplastics in total, of which 34 (0.49%) were spheres, 4313 (62.07%) were fragments, 2593 (37.31%) were fibers and 9 (0.13%) were films (Figure 5). In all tissue samples examined, all types (fragment, fiber, sphere and film) were present, but the distribution varied by tissue organ (Figure 8). Fragments and fibers are the most prevalent MP forms in marine ecosystems, with fragments often outnumbering fibers [39,40]. MPs fragments may originate from plastic disposal items connected to tourist activities, such as discarded plastic that breaks into small pieces, while the presence of fibers may be related to untreated wastewater that is dumped into coastal areas and waste from fishing activities [41]. This is consistent with other research on penaeid shrimp, including *M. monoceros*, *Penaeus monodon*, *F. indicus*, *P. hardwickii*, *Metapenaeus affinis* and farmed *L. vannamei*, as well as the prevalence of fibers discovered in *L. vannamei* from the HC lagoon [21,22,43,44]. Because larger plastic particles in the environment are unlikely to be ingested by shrimp due to their larger sizes [45] and are unable to degrade into smaller pieces rapidly, a lot of films and spheres in this study might not be able to be discovered in shrimp tissues. Fibers, on the other hand, are considerably smaller and might be easier to ingest.



Figure 4. Typical shapes, colors, and size of microplastics in tissue of shrimp.

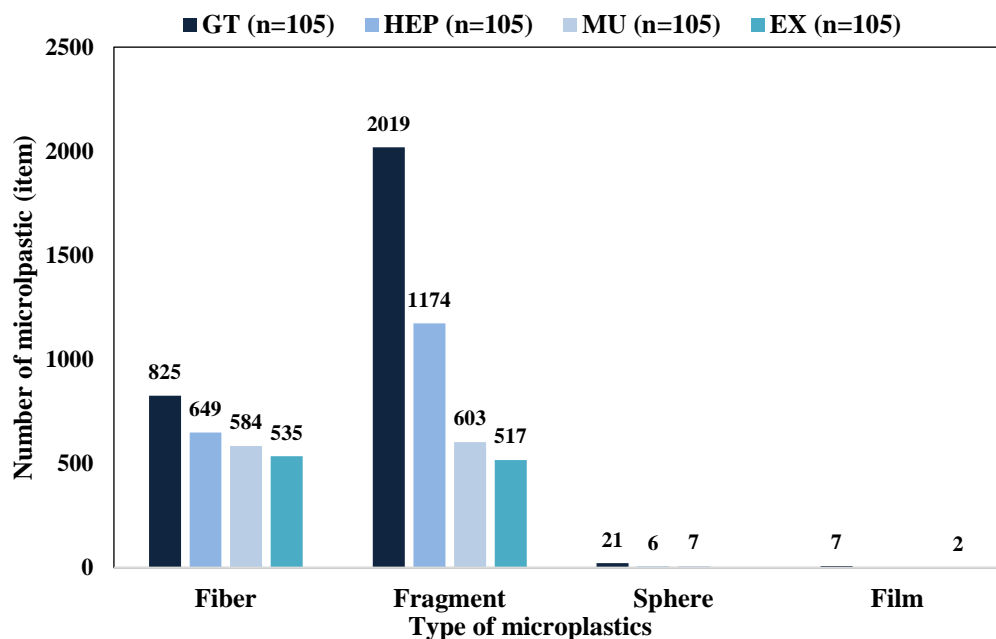


Figure 5. Summary of microplastic type in the gastrointestinal tract (GT), hepatopancreas (HEP), muscle (MU) and exoskeleton (EX) of *L. vannamei* (n = 105) in the seven ponds.

Under investigation, MP particles isolated from the *L. vannamei* tissues were detected in seven different colors: violet, red, transparent, green, black and pink. The most common color, with a total of 3036 items, was blue (43.69%), followed by red (23.86%, 1658 items), white/transparent (18.88%, 1312 items), violet (11.25%, 782 items), black (2.09%, 145 items), pink (0.22%, 15 items) and green (0.01%, 1 item) (Figure 6). The three colors that were most common in the tissues were blue (43.69%), red (23.86%) and white/transparent (18.88%) (Figure 6). Common findings in MPs' observations include the presence of fibers and these colors, which have been attributed to urban waste, damaged clothing (laundry) and fishing nets, ropes and lines. According to Curren et al. [36], the white or transparent color of MPs is the predominant color of the shrimp *L. vannamei* from Malaysia and Ecuador and *M. afnis* from the Northwest Persian Gulf [43]. MPs have been identified in a variety of species, including decapod crabs. Table 2 provides a summary, including the MP uptake by shrimp penaeid species from various regions. The color black has been observed the most frequently in other locations and shrimp species, followed by blue and red [44].

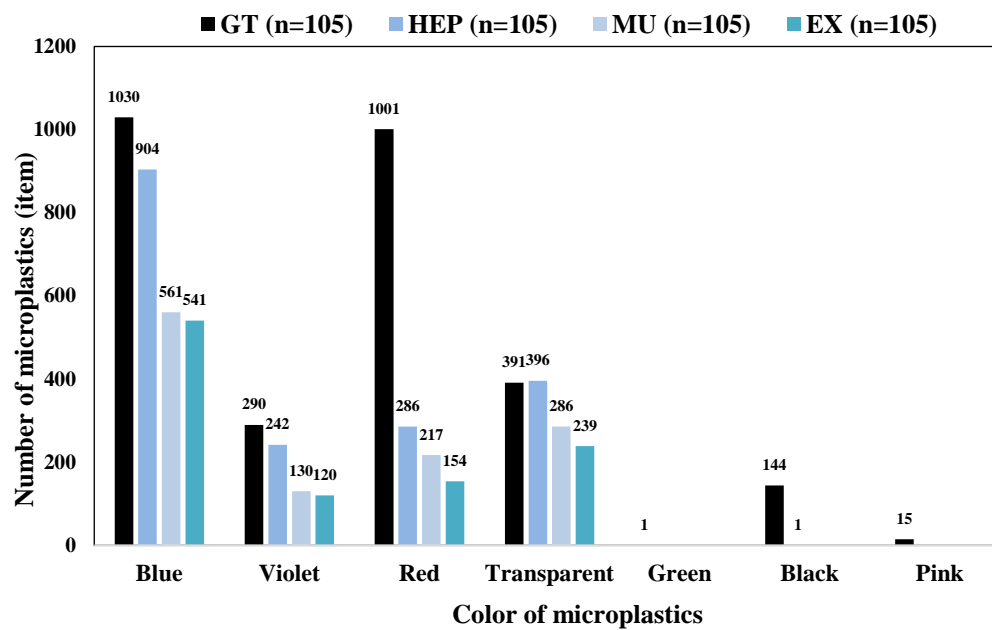


Figure 6. Summary of microplastic color in the gastrointestinal tract (GT), hepatopancreas (HEP), muscle (MU) and exoskeleton (EX) of *L. vannamei* (n = 105) in the seven ponds.

Table 2. Microplastic abundance and prevalence in shrimp penaeid species (MU, muscle; EX, exoskeleton; GT, gastrointestinal tract; GI, gills; HEP, hepatopancreas; ---, not available).

Species	Location	Tissues	Abundance Individual	Items/g (ww)	Dominant MPs Type	Color	Size (µm)	References
<i>P. monodon</i>	Bay of Bengal	GT	6.6±2.0	3.40±1.23	Fibers	Black	1000–5000	Hossain et al. (2020)
<i>M. monoceros</i>	Bay of Bengal	GT	7.8±2.0	3.87±1.05	Particles	Black	250–500	Hossain et al. (2020)
<i>F. indicus</i>	Coastal waters off Cochin, India	GT	0.39±0.6	0.04±0.07	Fibers	Red and blue	500–600	Daniel et al. (2020)
<i>P. hardwickii</i>	Xiangshan Bay, China	MU	0.95±0.28	0.25±0.08	Fibers	---	500–100	Wu et al. (2020)
<i>M. monoceros</i>	North eastern Arabian Sea	GT	7.2±2.6	78.5±48.4	Fibers	Black	100–250	Gurjar et al. (2021)
<i>P. stylifera</i>	North eastern Arabian Sea	GT	5.4±2.8	64.8±24.6	Fibers	Black	100–250	Gurjar et al. (2021)
<i>P. indicus</i>	North eastern Arabian Sea	GT	7.4±2.6	47.5±38.0	Fibers	Black	100–250	Gurjar et al. (2021)
<i>P. hardwickii</i>	Hangzhou Bay, China	GT, GI	2	7.8	Fibers	---	500–1000	Wang et al. (2020)
<i>M. affinis</i>	Northwest Persian Gulf	GT	---	1.02	Fibers and film	White or transp.	500–1000	Keshavarzifard et al. (2021)
<i>F. indicus</i>	Indonesia, Eastren Indian ocean	GT	---	5570±100	Spheres	Opaque	10–20	Curren et al. (2020)
<i>L. vannamei</i>	Ecuador	GT	---	13±1	Films	Transp.	---	Curren et al. (2020)
<i>L. vannamei</i>	Malaysia	GT	---	21±4	Films	Transp.	---	Curren et al. (2020)
<i>L. vannamei</i>	Gorgan Bay, Caspian Sea	GT	---	5.7	Fibers	Black	100–500	Bagheri et al. (2020)
<i>L. vannamei</i>	Shrimp farm, Guangdong Province, China	GT	6.3±2.4	14.1±5.7	Fibers	Blue	<500	Yan et al. (2021)
<i>L. vannamei</i>	HC lagoon, Mexico	GT	3.5±0.3	114.7±33.2	Fibers	Transp.	251–500	Valencia-Castañeda et al. (2022)
<i>L. vannamei</i>	HC lagoon, Mexico	GI	4.5±0.4	13.7±5.3	Fibers	Transp.	251–500	Valencia-Castañeda et al. (2022)
<i>L. vannamei</i>	HC lagoon, Mexico	EX	5.3±0.8	3.0±0.5	Fibers	Transp.	251–500	Valencia-Castañeda et al. (2022)
<i>L. vannamei</i>	farmed in northwestern Mexico	GT	261.7±84.5	7.6±0.6	Fibers	Transp.	30–2800	Valencia-Castañeda et al. (2022)
<i>L. vannamei</i>	farmed in northwestern Mexico	GI	13.1±1.8	6.3±0.9	Fibers	Transp.	30–2800	Valencia-Castañeda et al. (2022)
<i>L. vannamei</i>	farmed in northwestern Mexico	EX	2.6±0.6	4.3±0.9	Fibers	Transp.	30–2800	Valencia-Castañeda et al. (2022)
<i>L. vannamei</i>	Shrimp farm, Thailand	GT	27.36±2.28	137.78±16.48	Fragments	Blue	<100 to > 500	This study
<i>L. vannamei</i>	Shrimp farm, Thailand	HEP	17.42±0.90	16.31±1.87	Fragments	Blue	<100 to > 500	This study
<i>L. vannamei</i>	Shrimp farm, Thailand	MU	11.37±0.60	16.31±1.87	Fragments	Blue	<100 to > 500	This study
<i>L. vannamei</i>	Shrimp farm, Thailand	EX	10.04±0.52	4.37±0.27	Fragments	Blue	<100 to > 500	This study

The result of the size of MP assessments showed that the highest MPs in all tissues were measured at the size of 200–250 μm (29.90%, 2078 items), followed by >100 μm (29.31%, 2037 items), 250–500 μm (23.37%, 1624 items) and >500 μm (17.41%, 1210 items) as the lowest MPs (Figure 7). The size of MPs found in GT was less than 100 μm (31.72%, 911 items), followed by 200–250 μm (30.50%, 876 items), 250–500 μm (25.70%, 738 items) and >500 μm (12.08%, 347 items). The size of MPs found in HEP was less than 100 μm (40.04%, 736 items), followed by 200–250 μm (26.79%, 490 items), 250–500 μm (18.32%, 335 items) and >500 μm (14.65%, 268 items). MPs found in MU had a frequency size of 200–250 μm (31.49%, 376 items), followed by > 500 μm (26.21%, 313 items), < 100 μm (21.94%, 262 items) and 250–500 μm (20.35%, 243 items). MPs found in EX had a frequency size of 200–250 μm (31.88%, 336 items), 250–500 μm (29.222%, 308 items), > 500 μm (26.76%, 282 items) and < 100 μm (12.14%, 128 items) (Figure 7). The omnivorous shrimp *L. vannamei* can ingest pellets that range in size from 700 to 3000 μm , though juvenile shrimp prefer pellets that are between 124 and 210 μm in size [44]. As a result, the adult shrimp under investigation accumulated MP particles predominantly with a size between < 100 and > 500 μm . As bottom feeders, shrimps may have a higher chance of exposing the MPs in the sediment. They utilize a large variety of chemoreceptors (antennules) to sense food [46]. However, further study is necessary to completely comprehend the MPs (and food) that shrimp select in various environments.

The dominant sizes detected in the various tissues of *L. vannamei* from the area of shrimp activities in this study are comparable to those reported for *M. monoceros* (250–500 μm) from the Bay of Bengal [22] and *L. vannamei*, a farmed shrimp (< 500 μm) from Guangdong Province, China [47]. The findings of this study indicated that MPs were found in the four tissues of *L. vannamei* that were examined (GT, HEP, MU and EX) from the seven ponds, with the GT showing the maximum abundance (137.78 \pm 16.48 items/g) with sizes ranging from < 100 to >500 μm . The presence and predominance of fragments (60.07%) and fibers (43.69%) identified in the four tissues, together with the predominant colors of blue, red and white/transparent, might be attributed to the shrimp culture pond receiving rural, semi-urban, agricultural and aquaculture discharges.

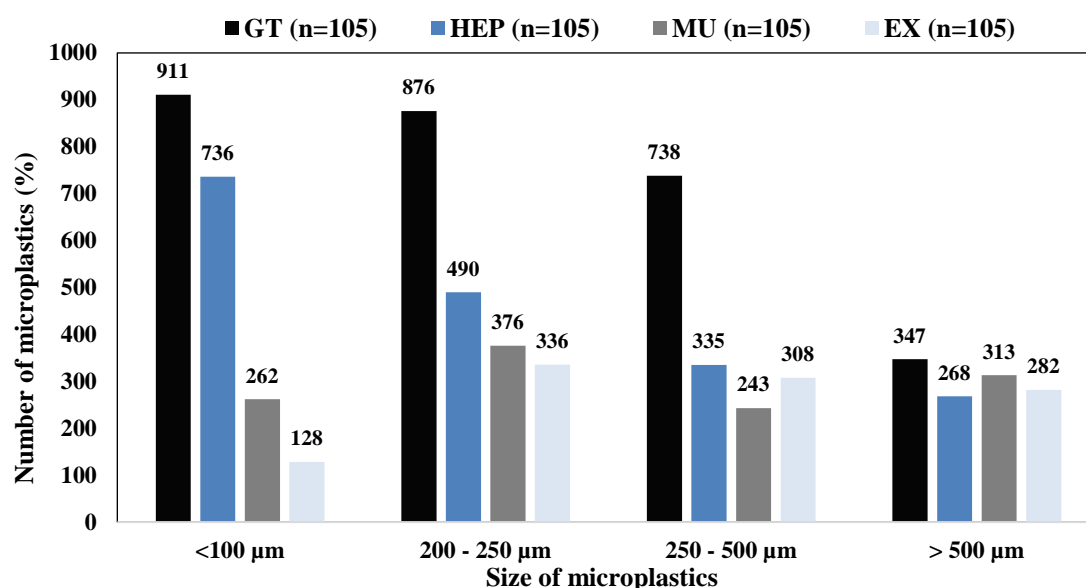


Figure 7. Summary of microplastic size in the gastrointestinal tract (GI), hepatopancreas (HEP), muscle (MU) and exoskeleton (EX) of *L. vannamei* (n = 105) in the seven ponds.

To grow, crustaceans shed their exoskeleton. During pro-ecdysis, a replacement cuticle develops underneath the older one and is later shed (ecdysis). Calcium is reabsorbed from the exoskeleton just before molting, softening the carapace so that it can be shed. The calcium that has been accumulated is then used to harden the new cuticle once ecdysis has taken place. Chitin, found that in crustacean exoskeletons, has the ability to bind metals [48]. Following uptake, these metals either bond to the inner exoskeleton matrix or, if they interact with the calcium in the exoskeleton matrix, may be absorbed into the exoskeleton's surface [48]. It was hypothesized that MPs, like metals, could calcify an animal's still-soft carapace after a molt and remain there until the next ecdysis. However, this approach needs to be investigated for MPs. There is little available data on MPs' impacts on shrimp. Duan et al. [49] discovered that MPs in *L. vannamei* affect the intestinal microbiota and the host's immunity, in contrast to Hsieh et al. [50], who reported that polyethylene MPs can affect antioxidant enzymes, increase lipid peroxide levels and cause tissue (midgut gland and gill) damage.

This study is the first to report the presence of microplastics in the tissues of Thai shrimps. Endocytosis and persorption are two mechanisms that explain how microplastics move from the gut to other tissues [51,52]. Particles as large as 10 μm have been found to enter the systemic circulation via endocytosis by M cells in the Peyer's patch of intestinal epithelium [53]. Persorption is the process by which larger particles up to 150 μm cross the intestinal barrier through the intercellular space of enterocytes [54]. The presence of microplastics of even larger size is explained by the agglomeration of smaller particles inside an organism's body [55], but the exact mechanism of such larger particle translocation is not well understood [56], as is the case with the larger size ($>500 \mu\text{m}$) of microplastic found in the muscle and exoskeleton of shrimp in this study. Furthermore, even after being rinsed twice with deionized water, large microplastics can still adhere to the joints of external appendages, including the swimming legs of this *L. vannamei* shrimp. Although many large MPs ($>0.5 \mu\text{m}$) were found in the muscle and skeleton of shrimp, the amount of this MP size was not significantly different in each shrimp tissue organ ($r = 2.234$, $P = 0.525$).

The Food and Agriculture Organization of the United Nations estimates that people may consume up to 53864 particles of microplastics of all types each year from seafood [57]. Based on the consumption of various fish and shellfish species that have been discovered to contain MPs, estimates of the amounts of MPs consumed by people have been made in a number of different nations. In order to estimate MP intake in humans, this study considered four factors: (i) the average consumption of Thai shrimp, (ii) the abundance of MP discovered in this study, which is typical of Thai shrimp consumption, (iii) the microplastics that remain inside the shrimp regardless of how they are cooked and (iv) accounting for both the least common scenario (shrimp consumed whole) and the most common scenario (when the GI and EX are discarded). The estimated intake was 66.18 and 28.79 MP item/shrimp/capita/day for the least and more common scenarios, respectively. The information raises awareness of the risks to human health associated with ingesting and being exposed to MPs. To completely understand the impact of MPs on human health when consuming seafood, more research is required. However, the implications for human food security, food safety and health are unclear [58].

The management of plastic waste upon inland and coastal areas can be helped by precise quantitative data on MP contamination. Due to the shrimp farming operations' close proximity to agricultural land and several nearby settlements, residues constitute a key source of MPs for the pond and are carried into the basin by wind and land runoff. This investigation provides background information on MP contamination in the farmed white leg shrimp, *L. vannamei*, which is helpful for future biomonitoring of MPs in a species of significant ecological and economic value.

3.2. MP polymer types in shrimp tissue organ

FT-IR analysis determined that 469 (63.64%) of the 737 randomly chosen particles were made of plastic, while 268 (36.50%) were found to be made of non-plastic materials. Of the 469 MP particles, 227 were made of polyethylene terephthalate (30.80%), 145 were made of cellulose acetate butyrate (19.68%), 68 were made of polyvinyl acetate (9.23%), 13 were made of poly(ethylene-glycol)-methyl-ether (1.76%), while 7 were identified as polyvinylidene fluoride and nylon (each 0.95%). Poly (acrylonitrile-co-butadiene) had one item (0.14%) (Table 3). Figure 8 shows the FT-IR spectra of all polymers.

Table 3. Polymer of microplastics identified by FT-IR.

Description	Number	Percentage (%)
Total particle measured (random selection)	737	100 ^a
Total polymer identified	469	63.64 ^b
Polyethylene terephthalate (PET)	227	30.80 ^c
Cellulose acetate butyrate (CAB)	145	19.68 ^c
Polyvinyl acetate (PVAc)	68	9.23 ^c
Poly (ethylene-glycol)-methyl-ether	13	1.76 ^c
Polyvinylidene fluoride (PVDF)	7	0.95 ^c
Nylon	7	0.95 ^c
Poly (acrylonitrile-co-butadiene)	1	0.14 ^c
Total non-plastic particle	268	36.50

Notes: ^aPercentage of analyzed MP particles; ^bPercentage of polymers in analyzed MP particles; ^cPercentage of MP polymer type.

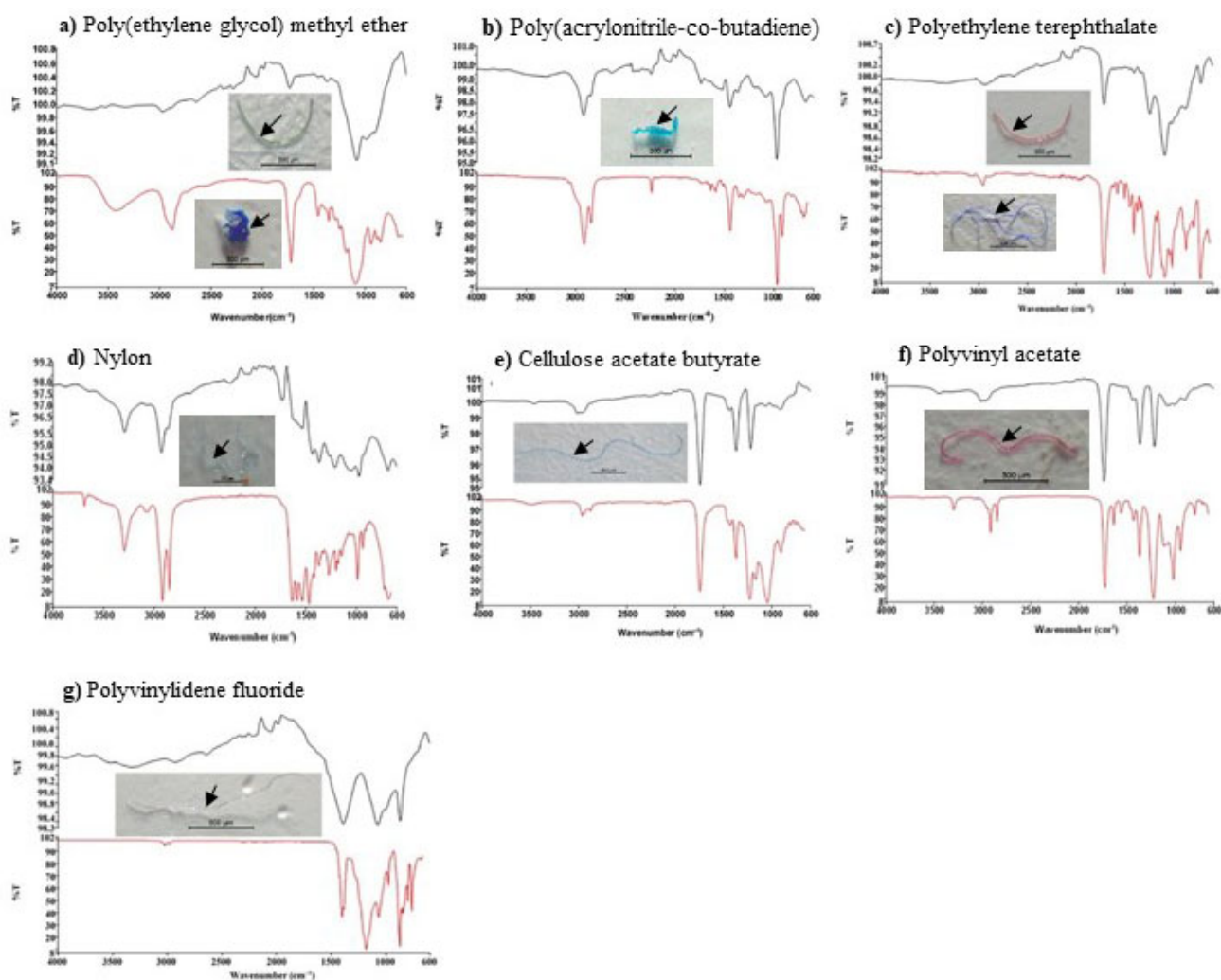


Figure 8. Photos of the most prevalent types of microplastics found in samples were analyzed with FT-IR: a) Poly (ethylene-glycol)-methyl-ether, b) Poly (acrylonitrile-co-butadiene), c) Polyethylene terephthalate, d) Nylon, e) Cellulose acetate butyrate, f) Polyvinyl acetate, g) Polyvinylidene fluoride.

3.3. Microplastic concentration in each pond surface water

All pond surface water samples contained 2282 items/5L of MPs, with an average of 65.2 ± 24.28 items/L and a range of 33–97 items/L in the study areas (Table 4). Among them, P3 had the highest concentration of microplastics (97 items/L), whereas P6 had the lowest concentration (33 items/L). The amount of microplastics in the environment is affected by the weather, ambient environment and anthropogenic activities [59,60]. The ponds' microplastic pollution may be in danger to increase due to the community's development. Shrimp aquaculture may be a significant source of microplastics. An increase in microplastic contamination may be caused by human activity near these ponds.

Table 4. A summary of the microplastic size, type and color found in 5L of each of the seven ponds.

Pond	<100 μm	200–250 μm	250–500 μm	>500 μm	Fiber	Fragment	Sphere	Foam	Blue	Violet	Red	White	Black	Yellow
P1	142	150	18	90	179	178		43	133	15	20	132	100	
P2	160	102	88	79	225	192	12		209	35	42	94	37	12
P3	112	138	150	85	287	198			250	3	83	118	20	11
P4	189	89	37	28	218	125			220	10	28	85		
P5	16	53	65	131	164	101			158	5	0	102		
P6	0	42	72	51	85	80			75	7	30	53		
P7	31	43	54	67	135	60			120	25	0	50		
Total	650	617	484	531	1293	934	12	43	1165	100	203	634	157	23

Previously, trawls, samplers, or sieves were used to collect microplastics from surface water. The results of this research were compared with the microplastic densities reported by the various sample methods in order to rule out the possibility that various sampling techniques could provide inconsistent results. Using the same method, these ponds have a higher concentration of microplastics than the Maowei Sea, which has a concentration of 4.5 ± 0.1 particles/L, the North Yellow Sea (545 ± 282 items/ m^3), the South China Sea (2569 ± 1770 particles/ m^3) and the Yangtze Estuary (4137.3 ± 2461.5 n/ m^3). Estuaries and coastal areas have higher quantities of microplastic since they are located nearer to commercial and residential areas than Nansha islands. Another significant source of microplastic pollution is mariculture zones, which increases their prevalence along the beach. In addition, the distribution of microplastics may be impacted by sea waves, winds and various sampling times [61].

3.4. Types, colors, and sizes of microplastics in pond surface water

The most frequent type of microplastic in all seven ponds was fibers (56.66%, 1293 items), followed by fragments (40.93%, 934 items), foam (1.88%, 43 items) and spheres (0.53%, 12 items) (Table 4, Figure 9). The main color of microplastics was blue (51.05%, 1165 items) (Table 4, Figure 10). Microplastics with a size of less than 100 μm (28.48%, 650 items) predominated in surface water samples (Table 4, Figure 11). In this study, blue fibers made up approximately 51.05% of the microplastics found in surface water. It has been reported that 11 blue fibers were found in fishing nets off the coast of Hong Kong. The fibers might easily flow past the sewage filter due to their small size [62]. These findings suggest that the main source of microplastics in all seven ponds may be residential sewage from humans. Additionally, fibers were frequently found in this study. A previous study suggested that human textile washing may be the source of plastic fibers in the ocean environment [63]. Fibers are widely used in fishing-related activities, such as the production of fishing nets and ropes. The findings indicated that microplastic pollution in all seven ponds may also be a result of shrimp farming operations.

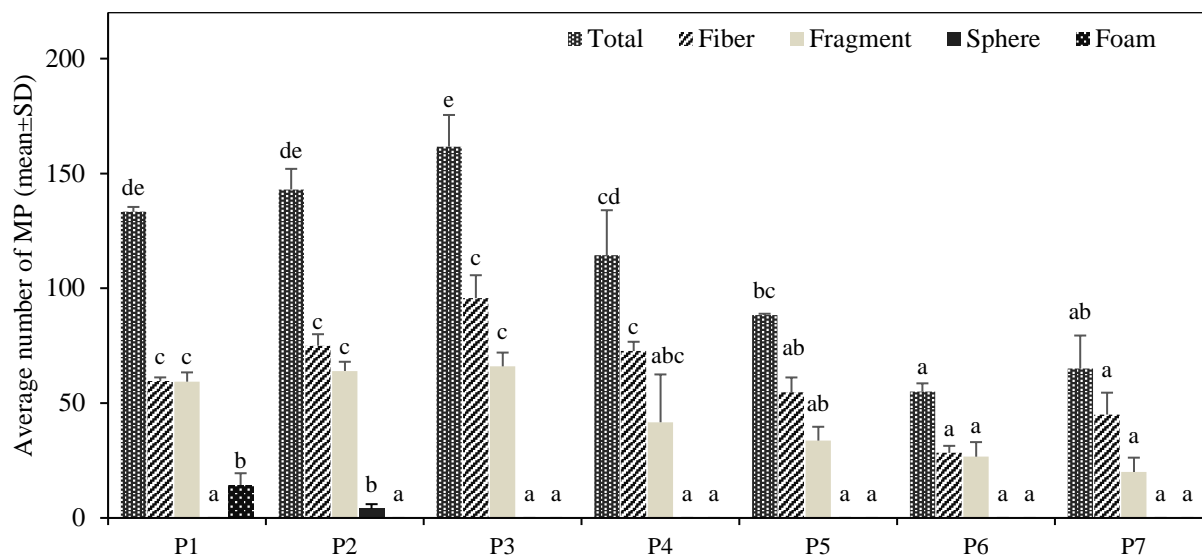


Figure 9. A bar graph shows the average number of MP (mean \pm standard deviation of the mean). Different superscript letters indicate significant differences according to one-way ANOVA and Tukey's post-hoc multiple comparisons test at $P < 0.01$.

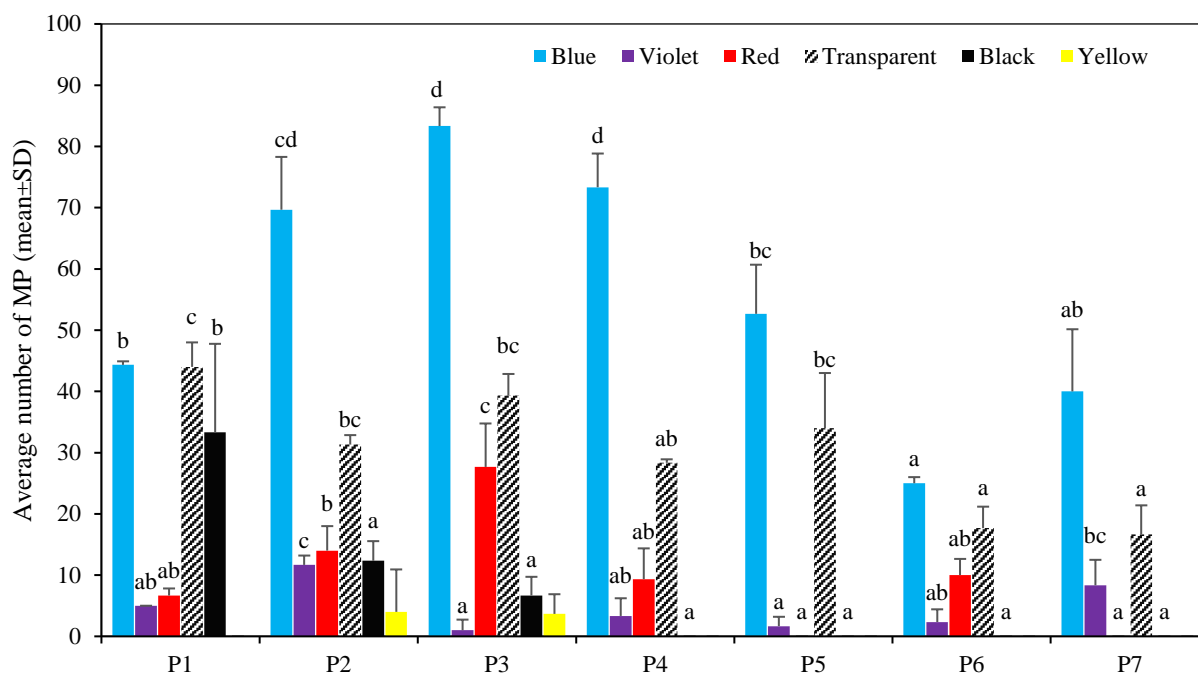


Figure 10. A bar graph shows the average number of MP (mean \pm standard deviation of the mean). Different superscript letters indicate significant differences according to one-way ANOVA and Tukey's post-hoc multiple comparisons test at $P < 0.01$.

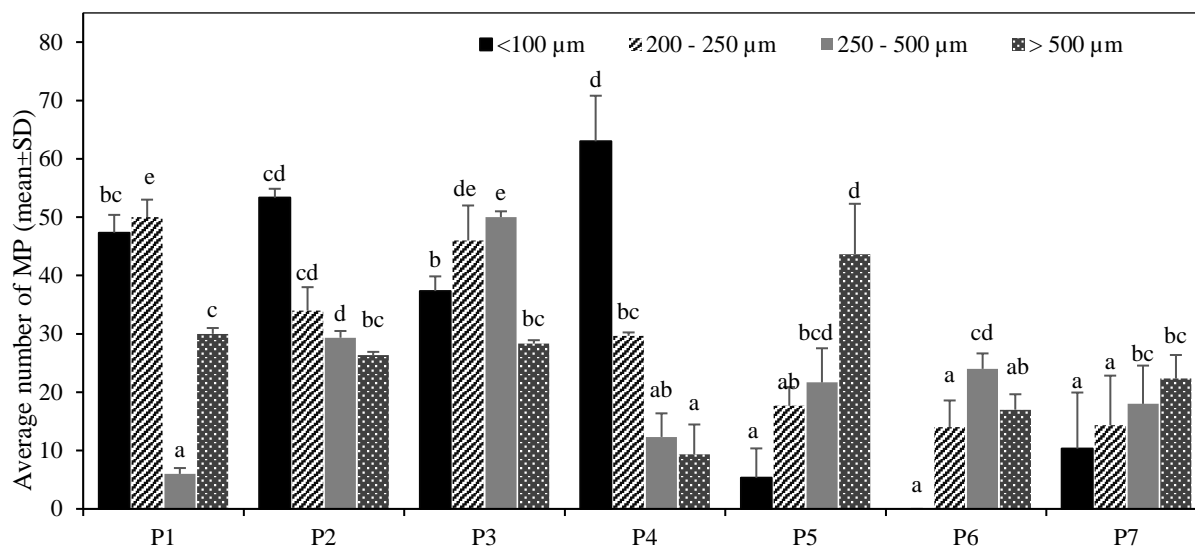


Figure 11. A bar graph shows the average number of MP (mean \pm standard deviation of the mean). Different superscript letters indicate significant differences according to one-way ANOVA and Tukey's post-hoc multiple comparisons test at $P < 0.01$.

3.5. Polymer types in pond surface water

Table 5. Polymer of microplastics identified by FT-IR of surface pond water.

Description	Number	Percentage (%)
Total particle measured (random selection)	304	100 ^a
Total polymer identified	187	61.51 ^b
Polyethylene terephthalate (PET)	77	25.33 ^c
Polyvinyl acetate (PVAc)	49	16.12 ^c
Cellulose acetate butyrate (CAB)	23	7.57 ^c
Polyethylenimine (PEI)	10	3.29 ^c
Trimethylolpropane tris[poly(propylene glycol) ether	13	4.28 ^c
Poly(tetrahydrofuran)	8	2.63 ^c
Polyethylene glycol	7	2.30 ^c
Total non-plastic particle	117	38.49

Notes: ^aPercentage of analyzed MP particles; ^bPercentage of polymers in analyzed MP particles; ^cPercentage of MP polymer type.

In this study, 304 randomly chosen items from a total of 2282 plastic items were subjected to FT-IR analysis (Table 5). The most prevalent polymer in the analyzed particles, as shown in Table 5, was polyethylene terephthalate (PET, 25.33%), which is frequently used for food and beverage packaging, especially for convenience-sized soft drinks, juices and water and was followed by polyvinyl acetate (16.12%), cellulose acetate butyrate (7.57%), trimethylolpropane tris[poly(propylene glycol) ether (4.28%) and polyethylenimine (3.29%). Other chemicals found also included poly(tetrahydrofuran) (2.63%) and polyethylene glycol (2.30%) (Figure 12). Cellulose acetate butyrate is commonly used as a binder and additive in coating applications for a variety of substrates, including plastics, textiles, metals and wood. Water-based (latex) paints contain polyvinyl acetate as a film-forming component. It is also utilized in adhesives. PET is a material that is frequently used to make fabrics, ropes, plastic

bottles, plastic bags and food containers [64,65]. The results suggest that anthropogenic waste can be a significant source of microplastics in the shrimp pond farming area.

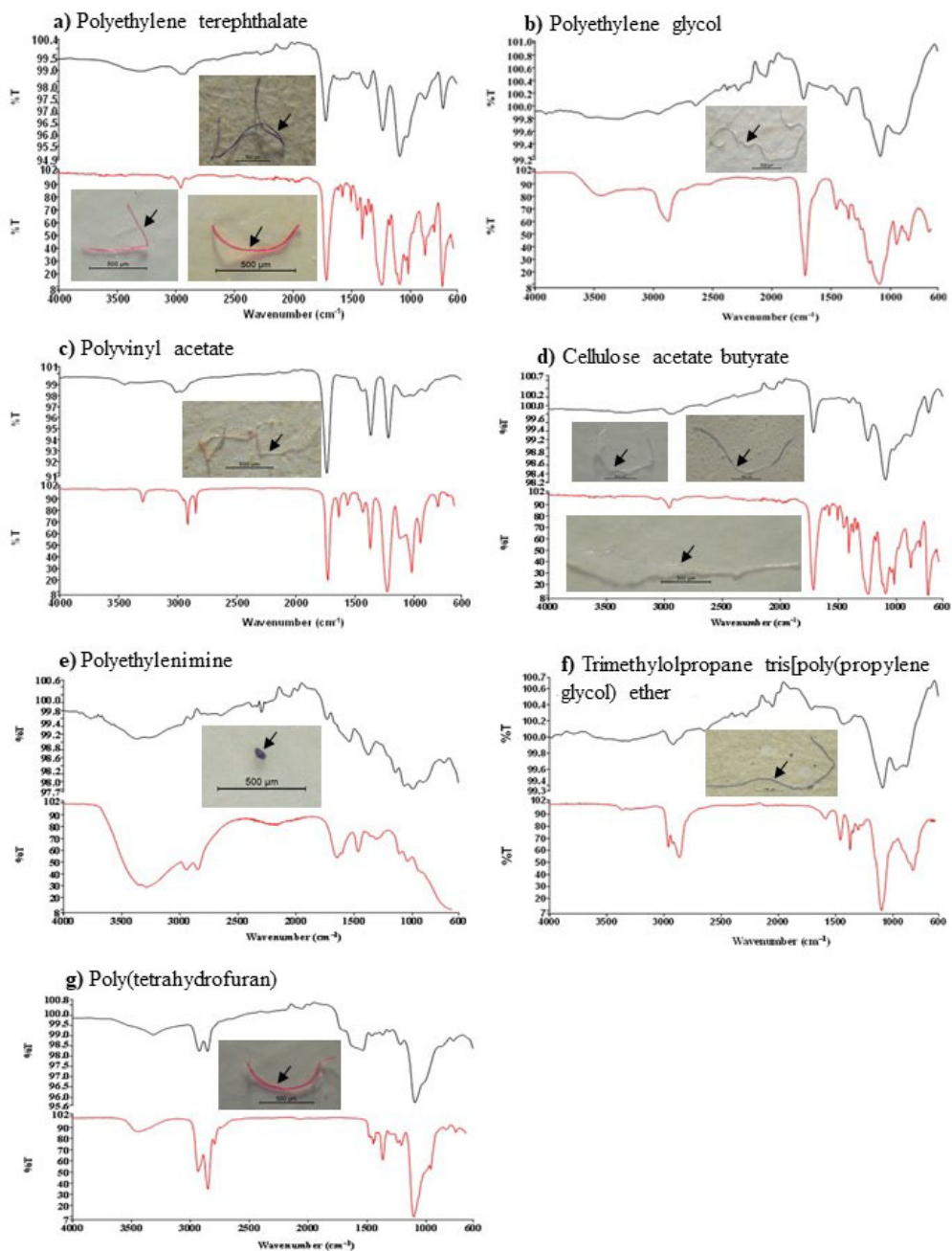


Figure 12. Photos of the most prevalent types of microplastics found in samples, along with FT-IR analyses: a) polyethylene terephthalate, b) polyethylene glycol, c) polyvinyl acetate, d) cellulose acetate butyrate, e) polyethylenimine, f) trimethylolpropane tris[poly(propylene glycol) ether, g) poly(tetrahydrofuran).

3.6. The correlation between the MPs in shrimp and pond water

The abundance of MPs found in farm-cultured organisms reflects the abundance of MPs found in the ecosystems where the organisms live and are handled. Because the feed, capture and storage of harvested shrimp are handled with plastic equipment and accessories, shrimp reared in farms have frequent contact with and possibly higher exposure to MPs. Shrimps may easily take particles suspected of being microplastics and store them in their tissues because of the abundance of MPs in the water. In this investigation, there was a negative correlation between the amount of microplastic in shrimp tissues and the amount of microplastic in pond water ($r = -0.778$, $P < 0.05$). The most common microplastic particles in the shrimp tissue organs and pond water were PET, PVAc and CAB.

4. Conclusions

In this investigation, MP contamination was found in 105 specimens of *L. vannamei* farmed shrimp that were collected from seven ponds in the Nakhon Pathom Province of Central Thailand. The MP particles were examined using four anatomical compartments: the gastrointestinal tract, hepatopancreas, muscle and exoskeleton. The limited sample size makes it impossible to establish definite conclusions, but the data analysis provides valuable information that can be used to plan future research. All individual shrimps that were investigated had MPs, with a calculated average of 66.17 ± 29.19 items per individual. The most frequent shape of a MP particle was fragment, with a fragment to fiber ratio of about 2:1. The majority of MP fragments and fibers were in the size range of <100 to $200\text{--}250$ μm . Each of the four anatomical compartments was contaminated differently depending on the size of the MPs. Overall, larger fiber-sized MP particles were found in the exoskeleton and gastrointestinal tract, whereas smaller fragment-sized MP particles were discovered in the muscle and hepatopancreatic tissues. According to the polymer type of MP particles found in *L. vannamei* individuals, MP contamination may originate from a wide range of sources, such as shrimp feeds, aquaculture water, fishing gear and rainfall. No individual shrimp was found to be MPs-free in this study, which emphasizes the need to monitor the pollution condition of the freshwater habitat and its species in order to inform and adjust waste management policy. It is reported for the first time that MPs contaminated edible parts of *L. vannamei*. While the muscular and exoskeleton sections contained the least number of MP particles, the gastrointestinal tract and hepatopancreas were revealed to be the most contaminated organs. However, the findings of this study suggest that MP contamination should be considered when preparing crustaceans for human consumption and when choosing sections to consume (e.g., avoiding the exoskeleton and gastrointestinal tract if possible) in order to reduce the risk of complications to human health. To confirm the results of this study, future work on this and other commercial species using other or comparable approaches is advised.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest.

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