ASSESSMENT OF MICROPLASTIC CONTAMINATION IN SOME MARINE SPECIES COLLECTED IN BA RIA - VUNG TAU PROVINCE

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Abstract

Recently, there has been increasing interest in studying microplastic pollution because of its dangerous effects on human health through the food chain. This paper presents the first results obtained on microplastic density in some marine species (including oysters, green mussels, mullet and tonguefish) cultured in Ba Ria - Vung Tau Province. The results indicated that microplastics were present in 100% of the analyzed samples with different concentrations in the order: oysters (10.3 ± 1.5 microplastics/individual) > blue mussels (7.4 ± 0.7 microplastics/individual) > mullet fish (4.1 ± 1.3 microplastics/individual) > tonguefish (1.2 ± 0.9 microplastics/individual). There are four main types of plastic found in the digestive organs of marine species including Polypropylene, Polyethylene Terephthalate, polyethylene, and polystyrene.

Keywords: *Ba Ria - Vung Tau, marine species, Microplastic contamination.*

ĐÁNH GIÁ Ô NHIỄM VI NHỰA TRONG MỘT SỐ LOÀI SINH VẬT BIỂN TẠI TỈNH BÀ RỊA - VŨNG TÀU

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Tóm tắt

Nghiên cứu về ô nhiễm vi nhựa đang ngày càng được quan tâm khi chúng có thể gây ra các vấn đề nguy hiểm cho sức khỏe của con người thông qua chuỗi thức ăn. Bài báo này trình bày các kết quả thu được về mật độ vi nhựa trong một số loài sinh vật biển (bao gồm hàu, vẹm xanh, cá đối và -cá lưỡi trâu) được nuôi trồng tại tỉnh Bà Rịa - Vũng Tàu. Kết quả chỉ ra rằng vi nhựa có mặt trong 100% các mẫu phân tích với nồng độ khác nhau theo thứ tự hàu (10.3 ± 1.5 vi nhựa/cá thể) > vẹm xanh (7.4 ± 0.7 vi nhựa/cá thể) > cá đối (4.1 ± 1.3 vi nhựa/cá thể) > cá lưỡi trâu (1.2 ± 0.9 vi nhựa/cá thể). Có bốn loại nhựa chính được tìm thấy trong cơ quan tiêu hóa của sinh vật bao gồm nhựa Polypropylene, nhựa Polyethylene Terephthalate, nhựa polyethylene và nhựa polystyrene.

Từ khóa: *Bà Rịa - Vũng Tàu, ô nhiễm vi nhựa, sinh vật biển.*

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1. Introduction

Plastics and products from plastics have changed people's life with its durability, convenience and low price, especially in a developing country like Vietnam. Plastics have become an indispensable part of modern life when it appears in almost everyday things like bags, food containers, drinks, household appliances, synthetic textiles, etc. Along with the dominance of plastics, plastic waste has caused extremely dangerous consequences for the environment and ecosystems. According to the statistics of the United Nations Environment Program published at the International Consulting Workshop on the Development of national action plan on marine plastic debris management in December 2018, every year, Vietnam discharged to the ocean from 0.28 to 0.73 million tons of plastic waste (~ 6% of the world total), ranked the 4th in the world, after China, Indonesia and the Philippines. Despite growing awareness of the dangers of this contamination, knowledge of the sources and pathways of marine plastics is still limited.

The plastic particles persist for a very long time in the environment due to its slow degradation process. In addition, the plastic particles can adsorb different contaminants (e.g., persistent organic pollutants - POPs, non-metals, additives/ monomers, and heavy metals) from the water and transfer them to the aqua-products like fish, bivalve organisms or salt, etc. (Clere et al., 2022; Azad et al., 2018; Bakir et al., 2014). Therefore, plastic particles in the environment can negatively impact the organisms, such as health risk associated with ingestion of the pollutants; or the microplastic indigestion could also decrease energy reserves, inhibition or reduction of feeding/filtering activity, disrupt the endocrine and reproductive systems, translocation to the circulatory system, and increase toxic load in smaller organisms. Finally, microplastics can reach the human organism through numerous types of plastic contaminated food, such us salt, fish, and mussels, etc. (Lee et al., 2014; Li et al., 2018; Dang, 2021).

Ba Ria - Vung Tau coastal Province has advantages such as a large water surface area, so it is very suitable for the exploitation, farming, and processing of seafood. In recent years, the seafood consumption market of Ba Ria - Vung Tau Province has grown strongly, not only on the domestic scale but also in the international market, thus improving the quantity and quality of seafood products in Ba Ria - Vung Tau Province needs attention. The presence of microplastics in seafood will be one of the threats to food quality and safety. Many recent studies have shown that microplastics have been found in various parts of marine species, in which, the digestion organs and gills are the highest accumulation of microplastics (Sun et al., 2017; Nam et al., 2018; Pan et al., 2021; Pham et al., 2022). However, studies on plastic pollution in marine species in Vietnam in general and in Ba Ria - Vung Tau Province in particular, are limited due to human resources, equipment, and funding. The objective of this study is to determine the abundance and nature of microplastic accumulated in some marine species (including oysters, blue mussels, mullet fish, and tonguefish) collected in Ba Ria - Vung Tau Province.

2. Methods and materials

2.1. Sample collection

In this study, samples of oyster, blue mussel, mullet and tonguefish were collected in Ba Ria - Vung Tau province. The sampling sites are shown in Figure 1. Oyster and mussel samples were collected directly from the farm in Long Hai town, Long Dien district, Ba Ria - Vung Tau Province. This is the largest oyster and mussel farming place of Ba Ria - Vung Tau Province. For the fish samples, mullet and tonguefish were purchased from Ben Dinh market, Ward 5, Vung Tau city. This is the largest wholesale seafood market in Vung Tau City.

Each organism sample was randomly selected from 5 to 10 adult individuals. Samples were collected in the most homogeneous size possible. For oyster and blue mussel samples, the weight of 10 individuals collected ranged from 80g - 100g/individual oyster, and from 40-50g/individual blue mussel. For the fish samples (7 individual mullet fish and 5 individual tonguefish), their weight varied between 120-150g/individual and 190-220g/individual for mullet and tonguefish, respectively. The samples were transferred to the laboratory and stored at 4-5°C (in refrigerated) until further analysis.

Figure 1. Sampling sites

2.2. Sample treatment

The extraction method and analysis of microplastic from marine species was based on the protocol of GESAMP (2019). The samples (oyster, blue mussel, mullet and tonguefish) were rinsed with filtered tap water, and the length/weight of each was recorded. The soft tissues were placed in a 1L conical flask and regarded as a replicate. Three replicates were used for each marine species. Next, 200ml of 30% H_2O_2 was added to each conical flask, the bottles were covered (with foil), and placed in an oscillation incubator at 65°C at 80rpm for 24h and then at room temperature for 24-48h depending on the digestion status of the soft tissue. The digestions were terminated once they appeared clear and no obvious particles were visible. A blank extraction (n = 3 replicates) without tissue (or seawater) was performed simultaneously to identify and characterize any procedural contamination.

A saturated saline solution (NaCl) was used to density separate the microplastics and other anthropogenic debris from dissolved liquid of the soft tissue via floatation (GESAMP, 2019). Approximately 800 ml of filtered NaCl solution was added to each bottle. The liquid was mixed and left to sediment overnight. The overlying water was gently removed and then filtered with GF/A glass microfiber filter paper. The filter paper was placed into a clean petri dish with a cover and was dried at 40°C for 12 hours prior to further microscopic and FTIR analysis.

2.3. Microplastic analysis

The filters were observed using a Leica S9i Stereo Microscope (range of magnification ×1000). The potential microplastic particles in the filter paper were counted and measured for physical characteristics (such as shape, size and color) using the measuring tool of the image analysis software LAS X (Leica Application Suite X): colors of microplastic were identified in 7 classifications: white, yellow, red, green, grey, blue and black; shapes of microplastic were identified in three categories; fragment, fiber, and bead; and size of microplastics was ranged between 100 - 5,000µm for fiber. All particles were photographed (Figure 2).

The nature of microplastics was determined using a FTIR-ATR iS50 Thermo Fisher Scientific® at Center for Analysis Service of Experiment in Ho Chi Minh city (CASE). The spectra were recorded as the average of 16 scans in the range of 4000 - 600cm^{-1} with a resolution of 4cm^{-1}. Spectra obtained were visualised in OMNIC 9.2.106 (Thermo Fisher Scientific Inc.), analysed, and compared against a self-generated library and the Hummel Polymer and Additives FTIR Spectral Library (Thermo Fisher Scientific Inc.). The polymers matching more than 70 % of reference spectra were accepted as suggested in previous studies (e.g. Lee et al., 2014; Pan et al., 2021; Pham et al., 2022).

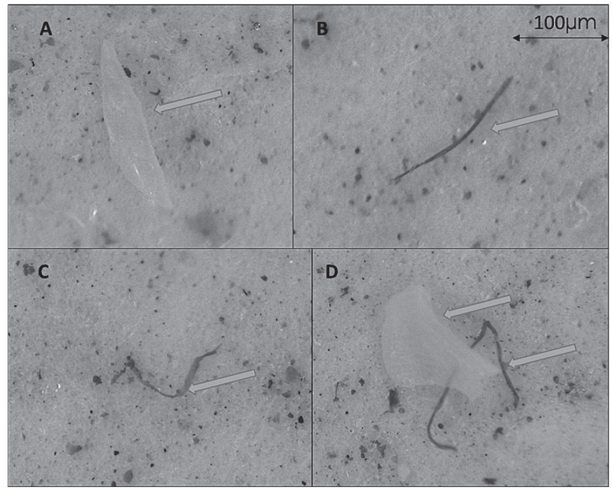
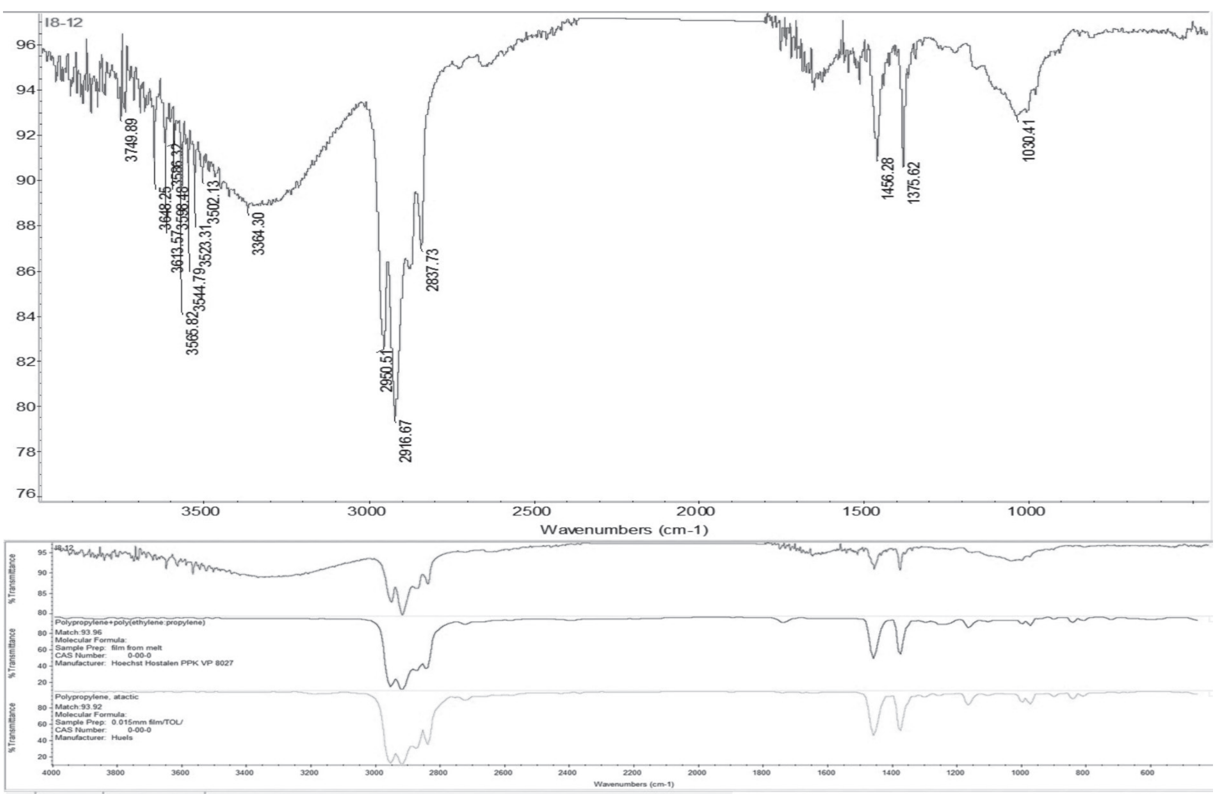
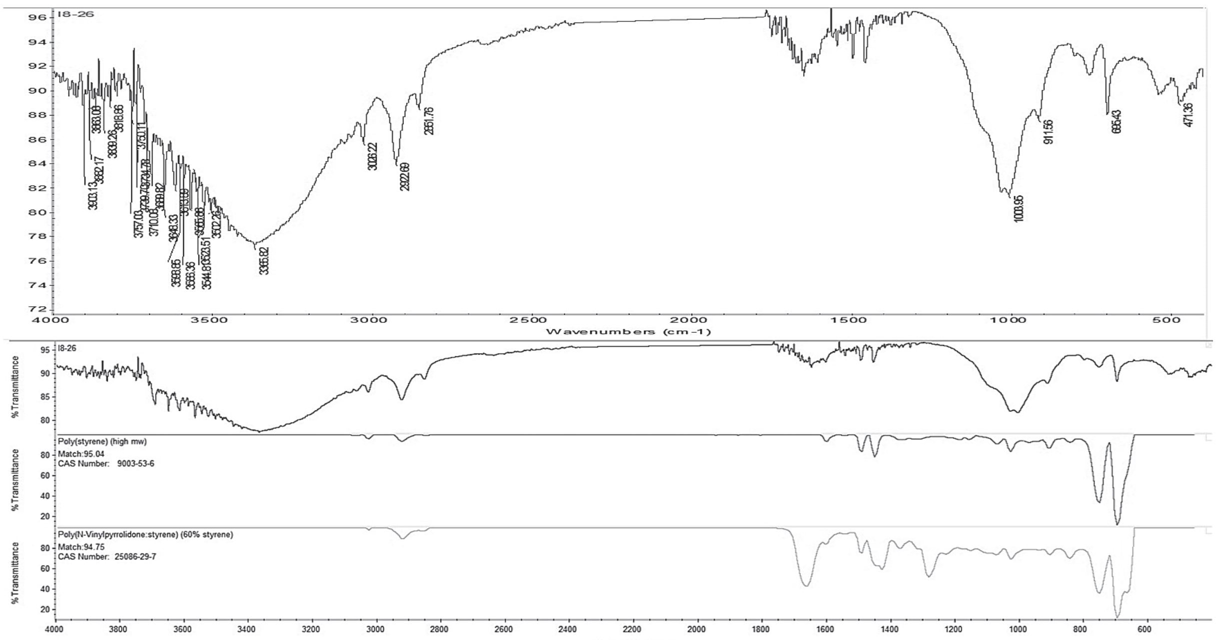


Figure 2. Images of different types of microplastic observed in marine species samples (×1000). Images A and D: fragment microplastic; Images B, C, and D: fiber microplastic.



	Index	Match	Compound Name
1	324	93.96	Polypropylene+poly(ethylene:propylene)
2	566	93.92	Polypropylene, atactic
3	41	93.91	POLY(PROPYLENE), ATACTIC
4	985	93.08	Poly(propylene), isotactic
5	5	92.98	Poly(propylene), isotactic
6	942	92.84	Polypropylene, isotactic
7	499	92.51	Poly(propylene:ethylene),83mol%C3
8	1653	92.28	POLYPROPYLENE-AMORPHOUS
9	1476	91.39	POLYPROPYLENE #3
10	437	91.35	POLYPROPYLENE #2

Figure 3. Examples of microplastic's FTIR spectra found in marine specie samples (e.g. Polypropylene and Polystyrene)



	Index	Match	Compound Name
1	322	96.18	Poly(styrene), monocarboxy terminated
2	322	95.87	Poly(styrene), monocarboxy terminated
3	255	95.43	Poly(styrene:allyl alcohol) (92-92.7% styrene)
4	321	95.26	Poly(styrene), dicarboxy terminated
5	266	95.15	Poly(N-Vinylpyrrolidone:styrene) (60% styrene)
6	44	95.04	Poly(styrene) (high mw)
7	266	94.75	Poly(N-Vinylpyrrolidone:styrene) (60% styrene)
8	323	94.16	Poly(styrene), monohydroxy terminated
9	321	94.01	Poly(styrene), dicarboxy terminated
10	277	93.95	Poly(2-acrylamido-2-methylpropanesulfonic acid:styrene) (95%

Figure 4. Examples of microplastic's FTIR spectra found in marine specie samples (e.g. Polypropylene and Polystyrene) (*cont.*)

2.4. Data processing

In this study, one-way analysis of variance (ANOVA) was used to test significant statistical differences between the abundance of microplastics of each salt type. Homogeneity and normality tests were applied to the data to validate the tests. All analyses were performed with a significant level of 0.05.

3. Results

3.1. Abundance of microplastic in marine species

The results obtained from the analysis of microplastics in marine specie samples (bivalve shells and fish) showed the microplastics are present 100% in the experimental samples with different abundance (ANOVA analysis results with 95% confidence level), and the abundance of microplastic in bivalve samples was higher than that in fish samples (Table 1). The abundance of microplastic in marine species collected in BaRia-VungTau province varied between 1.2 items/individual and 10.0 items/individual (Table 1) in the following order: oysters (10.3 ± 1.5 microplastics/individual) > blue mussels (7.4 ± 0.7 microplastics/individual) > mullet (4.1 ± 1.3 microplastics/individual) > tonguefish (1.2 ± 0.9 microplastics/individual). The different results on the abundance of microplastics accumulated in the marine organism samples can be explained by the characteristics of the food type and habitat of each species. If the mullet fish lives in the surface water with the main food of algae and zooplankton, the tonguefish lives in the bottom and their food is invertebrates. Therefore, mullet fish may mistake the suspended microplastic fragments in the aquatic environment as their food and consume them by mistake. For bivalve samples, the main food of oysters and green mussels are zooplankton and suspended particles in water. However, the weight of oysters is much larger than that of blue mussels, thus the amount of food consumed by oysters is more. This is the main reason for the abundance of microplastics accumulated in oysters more than that in green mussels.

Table 1. Abundance of microplastic (microplastics/individual) in some marine species collected in Ba Ria - Vung Tau Province

Samples	Mean	Min	Max
Oyster	$(10.3 \pm 1.5)^{a}$	7	14
Blue mussel	$(7.4 \pm 0.7)^{b}$	5	10
Mullet fish	$(4.1 \pm 1.3)^{c}$	2	8
Buffalo tongue fish	$(1.2 \pm 0.9)^{d}$	1	2

Note: Data were presented as mean ± standard deviation (SD). The letters a, b, c, and d indicate statistically significant differences at $p < 0.05$.

3.2. Size, shape, color, and nature of microplastics

The shape and color of microplastics accumulated in the both bivalve and fish sample groups were similar. In total of 182 microplastic observed, fibers were the predominant type of microplastic, corresponding to roughly 89% of the total microplastic. Fragment accounted for 11% of the total microplastic (Figure 5).

Microplastic occurred in marine organism samples with a variety of colors, including red, blue, white, grey, black, yellow and brown (Figure 5). The most common colors of microplastic found in sample were blue (45%), white and brown colors accounted for about 23 and 12%, respectively. The other colors were only found at a small ratio (red - 2%, grey -5% and yellow - 5%).

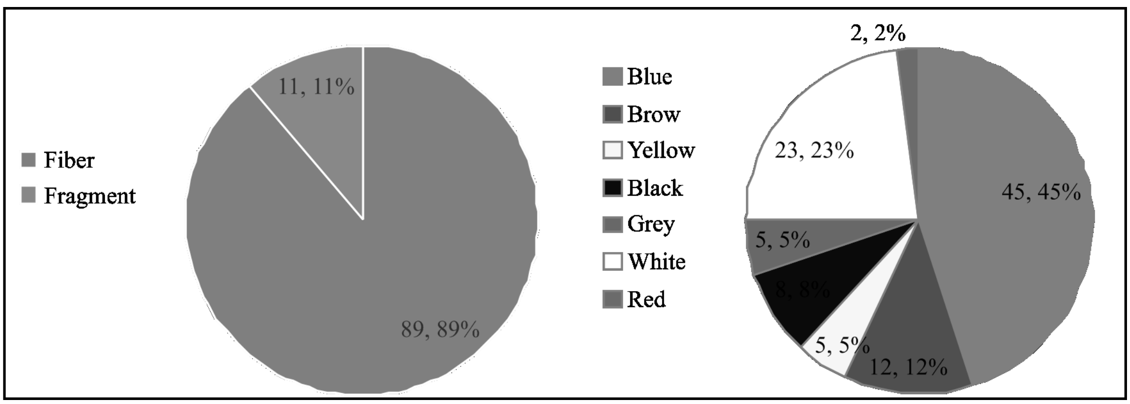


Figure 5. Contribution of the different shapes and colors of microplastic observed in marine species samples

In contrast to the shape and color, the size of the microplastic fibers in each group of marine organisms was significantly different (Figure 6). If the microplastic length observed in fish group ranges from 100-1000 µm with the predominant length of 800-1000 µm (accounted for 50% of total microplastic observed); for the bivalve group, the most frequent size of microplastic was in the range of 600-800 µm (~ 40% of total microplastic, Figure 6). For both bivalve and fish groups, microplastic fibers with sizes less than 200 µm and greater than 1000 µm were only found at a small ratio (Figure 6).

Finally, based on FTIR results, we detected four different polymer types, including polypropylene (PP), Polyethylene Terephthalate (PET), polyethylene (PE), and polystyrene (PS), among which, the most common is PP (accounting for 58% of the total microplastic), followed by PET accounting for 21% of the total. Polyethylene and polystyrene plastics accounted for 15% and 6% of the total microplastic, respectively. These are the types of plastic used very commonly in Vietnam to make plastic products such as packaging, bottles, food containers, drinks, household appliances, synthetic textiles, etc. In the aquatic environment, microplastics are suspended for a long time due to their durability and lightweight, thus the planktivorous organism may mistake microplastics for food. More seriously, the microplastics can adsorb on the surface a large number of pollutants such as heavy metals or persistent organic compounds. Meanwhile, the organisms related to the food chain will become a mediator of the pollutant transmission from the environment to the human body.

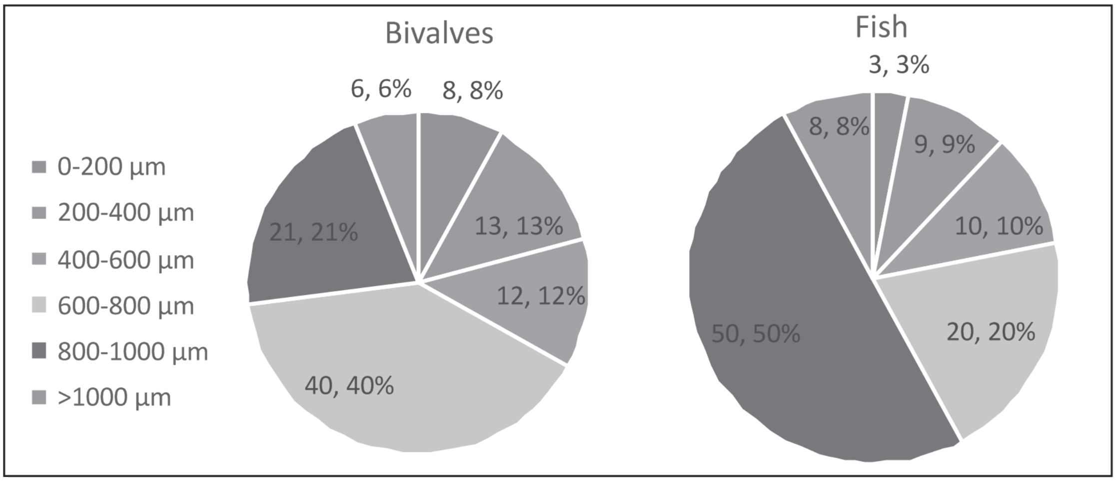


Figure 6. Size distribution of fiber microplastic in the marine species samples

Despite the limited number of marine organism samples collected and analyzed in this study, the comparison with other studies allows preliminary assessment of the microplastic contamination level in seafood of Ba Ria - Vung Tau Province. The results obtained from this study showed that the abundance of microplastic in some marine species collected in Ba Ria - Vung Tau Province is higher than that measured in Can Gio or Thanh Hoa (Table 2), but lower than that observed in Da Nang (33.25 items/individual oyster, Nam et al., 2019). Previously published literature on the abundance of microplastics in seafood showed a significant correlation between microplastic abundance and plastic emissions/microplastic pollution levels in surrounding seawaters (e.g. Tran-Nguyen et al., 2022; Dang, 2021; Strady et al., 2020). In fact, research by Tran-Nguyen et al., (2020, 2022) reported that the microplastic abundance in water environment (surface water and sediment) in the coastal area and estuary in Da Nang city was mostly higher than those measured in other regions of Vietnam. Moreover, Table 2 also showed that the abundance of microplastic measured in seafood of Vietnam is similar to that observed in China or Malaysia, but higher than that measured in Thailand, UK, Canada or New Zealand. For example, the microplastic abundance of oyster samples from Canada was 1.33 items/individual, from China is 8.3 items/individual fish. Finally, the most abundant types of microplastics observed in marine organism samples in the world are PP, PE and PET (Table 2). This result completely coincides with the previous studies (e.g. Waring et al., 2018; Strady et al., 2020; Dang, 2021), which showed that the most abundant microplastics observed in the marine environment were PE, PP, and PET.

The presence of microplastics in all marine organism samples of this study clearly showed the risk of microplastics being introduced directly into the human body through the consumption of oysters, blue mussels, or fish. After ingesting these plastic particles, the plastic particles less than 150 µm will pass through the gastrointestinal epithelium, in which the very small particles (<20 µm) will penetrate the cell membrane into internal organs and can even penetrate the walls of blood vessels. Although more than 90% of plastic particles can be eliminated through the human excretory system, plastics are non-biodegradable substances and bioaccumulation, thereby causing harmful effects on the immune system and cellular health of the human body (Cox et al., 2019). Research by Hwang et al. (2019) showed that there are 9 types of plastic particles found in the human body, the most common are PP and PET plastic, which are commonly found in food and beverage packaging.

Table 2. Type and abundance of microplastic in some marine species in the world

Samples	Microplastics/ individual	Type of microplastic	Origin	References
Fish	8.3	PP, PE, PS	China	Pan et al, 2021
Fish	4-6.5	PE, PP	Malaysia	Foo et al., 2022
Fish	0.97-1.75	PET, PVC, PS	Thailand	Azad et al., 2018
Oyster	1.1-6-4	PET, PE, PP	UK	Li et al., 2018
Oyster	1.33	PE, PET, PP	Canada	Mathalon & Hill, 2014
Fish	2.5	PP, PE	New Zealand	Clere et al., 2022
Oyster	5.5	PE, PP, PS	France	Phuong et al., 2016
Oyster	33.25	PE, PP, PS	Da Nang, Vietnam	Nam et al., 2019
Clam	1.3	PET, PP	Can Gio, Vietnam	Thanh et al., 2022
Oyster	1.11	PE, PP, PS	Can Gio, Vietnam	Thanh et al., 2022
Green mussel	2.6	PP, PET	Thanh Hoa, Vietnam	Phương et al., 2018
Fish	1.3-9.3	PP, PET	Thanh Hoa, Vietnam	Phương et al., 2018
Oyster	10.3	PP, PET, PE, PS	Ba Ria - Vung Tau, Vietnam	This study
Blue mussel	7.4	PP, PET, PE, PS	Ba Ria - Vung Tau, Vietnam	This study
Fish	1.2-4.1	PP, PET, PE, PS	Ba Ria - Vung Tau, Vietnam	This study

Note: PA - polyamide, PE - polyethylene, PET - polyethylene terephthalate, PP - polypropylene, PVC - polyvinylchloride, PS - polystyrene

4. Conclusion

The results obtained showed that microplastics were present in 100% of the marine species samples (including oyster, blue mussel, mullet and buffalo tongue) collected in Ba Ria - Vung Tau Province. The abundance of microplastic accumulated in the digestive organs of each species varies, depending mainly on the habitat, feeding habits as well as body size. If the microplastic abundance accumulated in oyster samples were highest (10.3 ± 1.5 microplastics/individual), tonguefish had the lowest (1.2 ± 0.9 microplastics/individual). For both bivalves and fish samples, fibers were the predominant type of microplastics, accounting for more than ~90% of total microplastic particles, and blue was the predominant color of microplastics (~50%). In addition, four types of polymers were detected by FTIR, including polyethylene (PE), polypropylene (PP), polystyrene (PS), and Polyethylene Terephthalate (PET).

It is clear that the presence of microplastic in the food chain in general and seafood in particular, will be a threat to human health, as well as have a negative impact on the aquaculture, and the socio-economy of populations that subsist with their production. Human food safety and health need to be a priority not only for the scientific community but also for the government, aiming at developing appropriate measures and policies to reduce plastic pollution and the risks associated with plastic pollution./.

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