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Microplastic contamination in filter-feeding bivalves inhabiting the natural ecosystem of Da Nang Bay: An investigation of oysters (*Ostrea rivularis*) and green mussels (*Perna viridis*)

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ABSTRACT

Microplastic (MP) contamination in marine organisms has been observed globally, especially in bivalve species inhabiting estuarine and coastal environments. In this study, microplastics are present in bivalve animals (i.e., oysters (*Ostrea rivularis*) and green mussels (*Perna viridis*)) that grow and develop in the natural environment in the Da Nang Bay area. These seafood species are produced abundantly in the region and are preferred by both visitors and residents for their daily consumption. MPs are fully characterized through abundance, shape, size, and color. MPs were detected in 100% of the examined bivalves, with an average density of 3.5 ± 2.8 items/g tissue, above the average levels seen in seafood throughout Asia. The concentration of MPs in bivalve species in the region varies from 3 items/individual to 47 items/individual, with oysters averaging 11.0 ± 5.0 items/individual and mussels averaging 18.0 ± 8.8 items/individual. Mussels have a considerably higher accumulation of MPs compared to oysters. The major component of MPs found in filter-feeding bivalve organisms consists of black plastic particles smaller than 0.5 mm in size and in fibrous form. There is a notable difference in the size of MPs between oysters and mussels, with a greater prevalence of larger MPs in oysters compared to mussels. This is a caution for MP contamination in creatures inhabiting the Da Nang Bay region. Consequently, it is essential to promptly implement steps to mitigate microplastic contamination in the research region.

Keywords: Microplastic, bivalves, oysters, mussels, Da Nang Bay.

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INTRODUCTION

The ubiquitous presence of substantial amounts of microplastics (MPs) in the marine environment poses an important risk to the safety of marine organisms. Bivalve organisms have a broad distribution over many locations, possess filter-feeding skills, are often exposed to aquatic habitats and sediments, and play a crucial part in the food web. Consequently, they are evaluated as effective indicators for new environmental contaminants, including MPs [1–4]. Recent studies indicate that MPs have entered the bodies of bivalve mollusks globally in both natural habitats and farmed contexts [5–7]. MP particles have been detected in bivalve organisms inhabiting the polar areas of the Earth [8]. On the other hand, prolonged exposure to MPs may adversely affect the growth of filter-feeding bivalves, inducing oxidative stress and leading to the accumulation of toxic chemicals that damage the genes and proteins of these organisms [9–11].

Bivalve mollusks are one of the seafood species that constitute a large production in the coastal regions of Vietnam. They are favored by the local population for daily meals, and their demand has been increasing in recent years [12]. Plastic fibers have been detected in both canned commercial seafood [13] and fresh seafood available at supermarkets and marketplaces [14–16]. Due to their diminutive size, extensive surface area, and the additives employed in plastic manufacturing, MPs can transport, disseminate, and absorb toxic chemicals in the environment, including heavy metals and persistent organic pollutants (PAHs, PCBs, HCHs, DDTs) [17–19]. The presence of MPs in seafood hinders seafood consumption in some industrialized countries [20] and may impact human health via the food chain [16, 21]. Bivalve mollusks are a very nutritious category of seafood, often collected by local populations and significantly contributing to the lives of coastal people in Vietnam [22–24]. Da Nang Bay has a substantial density of bivalve mollusks [25], comprising 25.45% of the entire benthic fauna, with oyster density at 0.06 individuals/m² and green mussel density at 0.31 individuals/m² [26]. Wildly cultivated bivalve mollusks are seafood varieties preferred by both visitors and residents. Consequently, it is essential to evaluate the possible danger levels of developing environmental contaminants, especially MPs, to guarantee consumer safety concerning bivalve species.

Currently, research on the accumulation of microplastics in filter-feeding benthic animals in the coastal regions of Vietnam is very limited. Therefore, this study will contribute additional information and data on the presence, quantity, size, shape, and color of microplastics in bivalve species living in the natural environment of Da Nang Bay.

METHODOLOGY

Sampling locations

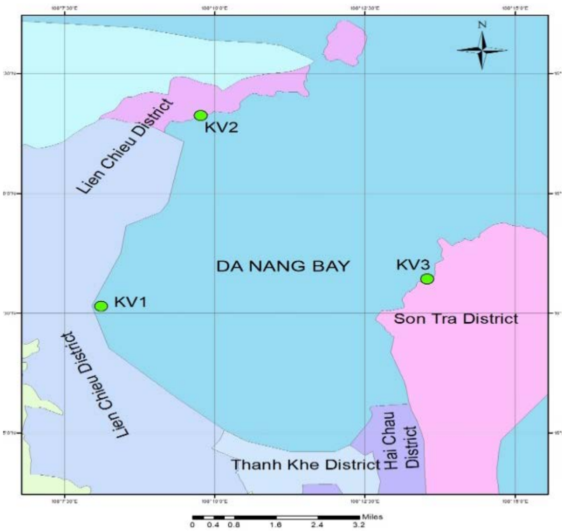

Figure 1. Sampling locations for oysters and green mussels at Da Nang Bay

In July 2023, samples of oysters (*Ostrea rivularis*) and green mussels (*Perna viridis*) were purchased from three locations where local inhabitants often trawl for these mollusks. Figure 1 contains the information on the sample collecting site. These locations are natural habitats for oysters and mussels. Among them, location KV1 is near the urban area, while locations KV2 and KV3 are pristine coastal areas, minimally affected by human activities. The sample collection was conducted by local people experienced in fishing and members of the research team. At each sample site, 20 individual oysters (≥ 80 mm) and 20 individual commercial mussels (≥ 50 mm) were collected. The weight and size of the sample individuals are provided in Table 1.

The bivalve filter-feeding animal sample was stored in a storage container with dry ice at about -5°C and brought immediately to the laboratory.

Table 1. Morphological parameters of bivalve samples

	Oysters			Green mussels		
	Size (mm)	Weight (g)	Tissue weight (g)	Size (mm)	Weight (g)	Tissue weight (g)
Min	89	66.94	5.02	50	10.03	2.01
Max	130	84.37	12.00	82	22.11	4.75
Mean ± SD	108 ± 11.9	74.13 ± 5.64	8.12 ± 1.93	65.6 ± 8.5	16.96 ± 3.28	3.20 ± 0.82

Microplastics analysis

The bivalve filter-feeding animals were cooled to room temperature, rinsed with distilled water, measured for body length using a ruler (Figs. 2a, b), and weighed separately. Carefully use a knife and scissors to dissect the flesh tissue of each specimen (Figs. 2c, d), weigh the flesh tissue of each specimen, and proceed with the subsequent stages to ascertain the properties of MPs.

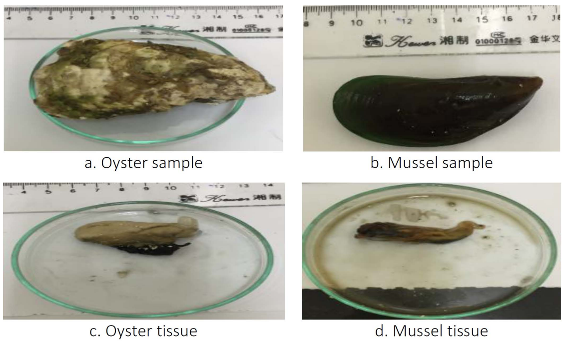

Figure 2. The morphology of the bivalve filter-feeding species

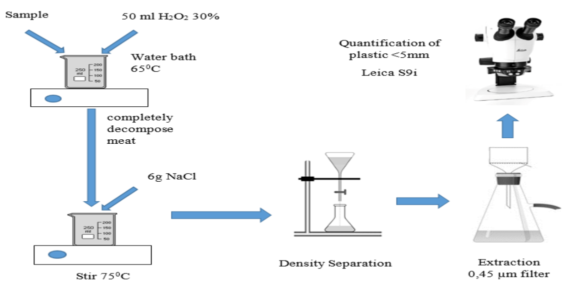

Figure 3. Flowchart of MP analysis in bivalves

The characterization of MP characteristics in filter-feeding bivalves is adapted from the approach [27] and suitably modified for laboratory settings, as seen in Figure 3.

Number, size, shape and color are observed directly on the Leica S9i stereo microscope with 6.1x-55x magnification connected to LAS-X image analysis software.

Controlling microplastic contamination in the laboratory

MP contamination in filter-feeding bivalve species is carefully studied in laboratory settings. The personnel operating the experiment wear white lab coats (100% cotton) and use disposable rubber gloves during the whole procedure. Thoroughly sanitize the whole environment and the experimental instruments with 70% alcohol before commencing the experiment. The experimental procedure for managing MP contamination from the air involved utilizing Petri dishes filled with distilled water, which was filtered through 0.45 µm filter paper. The presence of MPs in the water was subsequently examined using a stereomicroscope upon the conclusion of the experiment.

Data analysis

The variables depicting MP contamination in filter-feeding animals within the research region are as follows: MP density per individual refers to the quantity of MPs found in a single bivalve (items/individual); MP density per gram of flesh denotes the number of MPs in one gram of wet flesh (items/g); average MP density per individual is calculated as the mean number of MPs per individual ± standard deviation. The disparity in MP accumulation between oysters and mussels was assessed using SPSS statistical software (T-Test), $p < 0.05$; The association between the morphology of oysters and mussels and the extent of MP accumulation was analyzed using STATISTICA software (Correlations), $p < 0.05$.

RESULTS AND DISCUSSION

Microplastics distribution

A total of 1,746 MPs were identified in 60 individual oysters and 60 individual mussels across three locations in Da Nang Bay, as seen in Figure 1. MPs were detected in all samples of bivalve organisms within the research region. The density of MPs in each individual bivalve filter feeder varies from 3 to 47 items/individual. The average MP density in oysters is 11 ± 5 items/individual, whereas in mussels, it is 18 ± 8.8 items/individual.

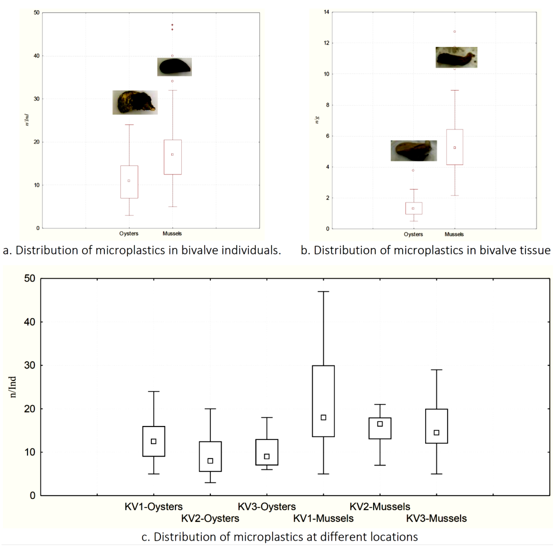

Figure 4. Microplastic distribution in bivalve animals in Da Nang Bay

Figure 4a illustrates the distribution of MP contamination levels in bivalve species at Da Nang Bay. The concentration of MP pollution in filter-feeding bivalves from Da Nang Bay is compared with that of bivalve species inhabiting other global bays, as shown in Table 2.

Table 2 indicates that MP contamination in filter-feeding bivalve species in Da Nang Bay is above levels seen in other global bay regions. The concentration of MPs per gram of tissue varies from 0.5 to 12.7 items/g, with typical densities of oysters and mussels measured at 1.4 ± 0.6 items/g and 5.7 ± 2.4 items/g, respectively, as seen in Figure 4b. The mean MP density in the muscle tissue of bivalve mollusks in the research region is 3.5 ± 2.8 items/g, beyond the average MP density in the muscle tissue of mollusks throughout Asia, which is 2.5 ± 2.9 items/g [28]. This may impact the habitat of filter-feeding bivalves (water, sediments, plankton) in regions exhibiting MP contamination. Consequently, it is important to establish monitoring and control methods, along with strategies to decrease MP pollution in the area, to minimize the effects on the aquatic ecology and the health of the local populace dependent on seafood from the region.

MP pollution in bivalve species at different sampling locations: The distribution of MPs in bivalve animals at different sampling locations in Da Nang Bay is presented in Figure 4c. In the KV1 area, which is directly affected by wastewater from urban residential activities along the coast and wastewater from inland flowing into the sea through the Han River and Cu De River, the level of MP pollution in both oysters and mussels tends to be higher compared to KV2 and KV3 (the sampling areas further from the shore). Furthermore, some studies have shown that MP pollution in bivalve species is influenced by the dynamic factors of their habitat, with individuals living in estuarine bays exhibiting higher levels of MP pollution compared to areas outside [4, 29]. The research results indicate that the level of MP pollution in bivalve species is significantly influenced by their habitat.

MP pollution in different bivalve species: The statistical calculation values comparing the average amount of MPs in oyster and mussel specimens, as well as the number of MPs per gram of fresh meat in oysters and mussels, both have a significance value (2-tailed) = 0.00 < 0.05, indicating a significant difference in the level of MP pollution between oysters and mussels, with a trend showing that the level of

MP pollution in mussels is higher than in oysters, similar to the published results [30] indicating a significant difference in pollution levels between oysters and mussels.

Table 2. The degree of MPs accumulation in bivalve species across some regions globally

Locations	Bivalve species	Distribution in each individual (items/individual)	Distribution in tissue (items/g)	Ref.
Bandon Bay, Thailand	Oysters	-	1.42	[31]
	Blood cockles	-	1.01	
	Green mussels	-	0.87	
Daya Bay, China	Scallop, mussel, oyster, clam	3.5 to 8.6	0.2 to 3.1	[32]
Hangzhou Bay, China	*Mactra veneriformis*	0.3 ± 0.18	0.83 ± 0.48	[33]
	Sinonovacula constricta	0.08 ± 0.04	0.67 ± 0.33	
San Francisco Bay	Resident bivalves (clams and mussels)	-	3.0 ± 2.4	[34]
Todos Santos Bay and San Quintin Bay, Mexico	Oysters	-	0.06 ± 0.02	[30]
Da Nang Bay	Pacific oyster (in Farming areas)	33.25 ± 25.93	33.25 ± 25.93	[35]
Da Nang Bay	Oysters	11 ± 5	1.4 ± 0.6	This study
	Mussels	18 ± 8.8	5.7 ± 2.4	

To clarify the level of MP pollution in oysters and mussels living in the natural environment of the surveyed area. The statistical correlation between MP density in individuals and MP density in tissue was examined with a value of ($p < 0.05$), with the obtained R values for oysters and mussels being 0.82 and 0.83, respectively. Thus, to assess the level of MP pollution in bivalve species, one can choose between two parameters: the density of MPs per individual or the density of MPs per gram of tissue (applicable to commercially processed, packaged, or canned seafood products).

Consider the morphological correlation (length, individual mass) of oyster and mussel specimens throughout the entire study area with the density of MPs in the individuals, with a statistical value of $p < 0.05$. The correlation coefficients between the length, individual weight, and MP density in individuals are 0.33 and 0.15, which are considered to have weak correlations; this issue is also highlighted in the study [36].

Microplastic characteristics

MPs found in seafood are usually small in size (< 1 mm), accounting for over 90% of the total MPs present in organisms [37, 38]. MPs found in bivalve species in this study measured ≤ 3 mm (Fig. 5a). Among them, MPs with smaller sizes account for a larger proportion of both oysters and mussels. Specifically, the distribution rates of MPs in size ranges of 2–3, 1–2, 0.5–1, and < 0.5 mm for oysters are 5.3, 15.5, 29.5, and 49.7%, respectively; for mussels, they are 8.8, 17.8, 34.8, and 38.5%. Bivalve mollusks, including blue mussels, primarily feed on single-celled phytoplankton and organic detritus filtered through their gill system [39]. Fine particles are directed to the mouth, while heavier coarse particles fall to the edge of the gill and are expelled. Therefore, MPs in filter-feeding bivalve species are often small in size. The proportion of MPs with a larger size (≥ 0.5 mm) in oysters is higher than in mussels, while conversely, for smaller sizes (< 0.5 mm), the proportion of MPs in oysters is lower than in mussels. When comparing statistical values, the average size is $p < 0.05$, sig. (2-tailed) = 0 < 0.05, which means that there is a significant difference in the length of oysters and mussels, with the average length of oysters being 108 mm, larger than mussels (65.6 mm). This is precisely why oysters tend to accumulate larger MPs than mussels. Microplastics of smaller sizes were found to be more prevalent in bivalve mollusks, as indicated in the studies [35, 40] at aquaculture farms in Da Nang Bay. However, to understand the presence of MPs in the environment, it is necessary to monitor MPs in filter-feeding bivalve species of varying body lengths corresponding to different mouth sizes.

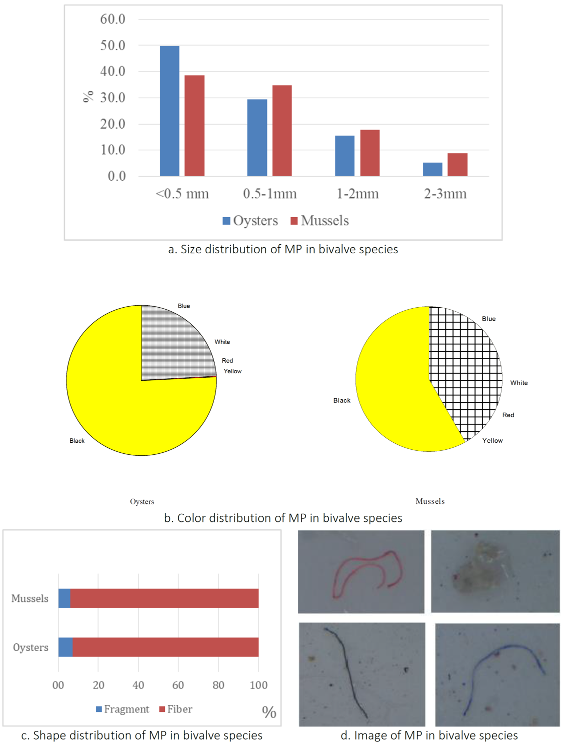

Figure 5. Characteristics of microplastics in bivalve species at Da Nang Bay

There are six colors observed in the MPs present in filter-feeding bivalves at Da Nang Bay, as shown in Figure 5b. Among them, black MPs account for the largest proportion, with oysters (75.9%) and mussels (58.2%); followed by blue MPs, with proportions in oysters and mussels being 14.5% and 22.5%, respectively; red, yellow, and white MPs make up a small percentage in bivalve species. There is no significant difference in the ratio of colors between oysters and mussels.

MPs in the form of fibers constitute the main component in samples of bivalve species [17, 41], with the proportion of fiber-shaped MPs ranging from 62% to 83% [37], and the fiber proportion exceeding 80% [37]. In this study, MPs were found in oysters and mussels in two forms: fibers and fragments, as shown in Figure 5c. In this, the proportion of fiber-shaped MPs constitutes the main component, with 92.8% for oysters and 93.9% for mussels; the remaining percentage consists of fragments, which account for 7.2% for oysters and 6.1% for mussels. The image of some identifiable MPs in bivalve animals living in the natural environment of Da Nang is shown in Figure 5d.

The risks of microplastics to the ecosystem

Microplastic pollution in the marine environment in Vietnam has been increasingly warned about [42, 43]. Studies have demonstrated the presence of microplastics in marine organisms in various regions of Vietnam, notably in filter-feeding bivalves [44], spreading through the food chain and affecting human health [45].

Plastic debris has also been proven to absorb and transport harmful organic substances to the environment and ecosystems, such as PAHs, PCBs, HCHs and heavy metals [14, 18, 46, 47]. Plastic particles with small sizes (< 150 µm) can be ingested by marine organisms and transported to the lymph nodes, reaching other organs of the organism [48]. Studies also demonstrate that bivalve species are affected by MPs due to their limited mobility and can easily absorb MPs through the filtering process [49]. The results of the study show that 100% of the bivalve samples contained microplastics; small-sized microplastics (< 1 mm) accounted for a large proportion (49.7% in oysters and 38.5% in mussels), with fibers being the most prevalent form (> 90% in both oysters and mussels). Therefore, the presence of microplastics in the study area's environment poses potential risks to marine organisms and human health. Additionally, some studies have shown that different types of microplastic polymers carry varying levels of risk (also known as the chemical risk of MPs) [50, 51]. However, in this study, the polymer properties of the microplastics present in the bivalve filter-feeders have not been characterized to clearly reflect the risk of microplastics to organisms in the study area, and further research is needed in future studies.

CONCLUSION

MP pollution in bivalve species living naturally in the environment of Da Nang Bay has been surveyed and assessed. Microplastics were found in all collected bivalve samples in the study area, with the density of microplastics in individuals being higher than in other bays around the world and the average microplastic density in the tissue of individuals being higher than the average in Asia. There is a difference in the level of microplastic pollution at different survey sites. The density of microplastics in bivalves tends to be higher in locations near urban areas and river mouths, and lower in offshore areas or those heavily influenced by dynamic conditions such as waves and currents.

The morphology of microplastics in bivalves has been identified. Small-sized microplastics (< 1 mm), fibrous, black and blue in color, are predominant. The risk of microplastics in the study area's environment is warned when most of the bivalve filter-feeder samples contain microplastics with small sizes and fibrous forms. However, this study has not addressed the type of polymer, which is one of the chemical risk factors of microplastics, posing a significant risk to marine organisms and human health, and further research is needed in subsequent publications.

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