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Original Article

Characteristics of Microplastics in Bivalves of Rock Oysters and Sea Clams in the South Central Coast, Vietnam

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Abstract: Microplastics have emerged as one of the new substances causing environmental pollution, which is a growing concern because their nature can pose risks to human health and the environment. We analyzed characteristics of microplastics such as density, size, shape, and polymer types in natural rock oysters, *Saccostrea sp*, and sea clams *Venus sp* in the South Central Coast using Fourier Transform Infrared Spectroscopy (µFTIR) on a microscope Nicolet iN10 MX. The results showed that microplastics in *Saccostrea sp* and *Venus sp* present with density of 1 - 21 and 4 - 5 particles/individual, respectively. The average concentration of microplastics was about 0.25 - 1.59 particles/gram of fresh tissue weight. The size of microplastics ranged from 20 µm to more than 500 µm, mainly found in range of 50 - 150 µm. Commonly found microplastics shapes were fragment and fiber. There were ten polymer types found, of which the most common was polyethylene terephthalate (PET), accounting for 58%, and the popular nylon type with 10% of the total microplastics. In addition, some other polymers were also detected by FTIR technique, such as polypropylene (PP) contributing about 9% of the total microplastics, HDPE 30060M, and Melamine-urea-formaldehyde resin contributing 7%. In summary, this study investigated the

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presence of microplastics and assessed the risk of microplastics in bivalves that possibly affect human health (PHI index of 100.81).

Keywords: Microplastics, Characteristics, *Saccostrea* sp., *Venus* sp., Fourier Transform Infrared Spectroscopy (μFTIR), Nicolet iN10 MX, polymers.

1. Introduction

Plastic and plastic products have many advantages for human beings, such as compactness, durability, and usefulness. Therefore, plastic manufacture has increased exponentially, and the plastic consumption has been covered in almost all countries. However, plastic is a waste that decomposes slowly. Large plastic waste will be broken down under mechanical and natural impacts into tiny plastic particles less than 5 mm in size called as microplastics [1-3]. These microplastics can be exited under natural conditions, taking hundreds to thousands of years in decomposition [4-6].

Microplastics have emerged as a global concern due to their ubiquitous presence in soil and water environments. It is also a potential cause affecting the ecosystem in general and human health when it is not strictly controlled. Many research projects have shown that microplastics can be harmful to humans and living organisms, which affect the digestive system and cause health problems that can enter the food chain through food and drink. Nowadays, microplastics have been found in many species of organisms and seafood (plankton, bivalves, shrimp, fish, clams, oysters...) [7-11]. Using bivalves as bioindicators of microplastic pollution was studied, and bivalves were assessed as transporter of microplastics to humans when clams and mussels were chosen to study as bioindicators for microplastic pollution in the sediment and water in Qingdao, China [8]. In Vietnam, the occurrence and distribution of microplastics in bivalves and sediments were interested to researchers for building database of bioindicators from microplastic pollution [12, 13].

In today's modern society, the problem of microplastic pollution is becoming more and more interesting. Although this issue received a lot of attention, the number of research is still limited. Lack of equipments in the microplastic analysis methods has not yet been perfected due to many different analysis processes can lead to inconsistent data results. Hence, microplastic pollution is considered one of the emerging types of pollution in environmental objects, with risky impacts on human health and the ecosystem. The government needs to have resolutions to warn about microplastic pollution and gets recommendations for plastic waste management. Based on the severity of the microplastic pollution problem, this research was conducted to assess the presence of microplastics in terms of quantity, morphology, size, and diversity of microplastic polymers in some bivalve organisms, as a basis for warning about the dangers of microplastics to humans and the environment, and also contribute to the database on microplastic pollution to continue implementing future research projects. The area of the South Central Coast, Vietnam, is popular and convenient in economic development, as well as bordering the East Sea with many islands and chosen to study in this research. In this area, microplastic data in environment were limited. Therefore, the present research assesses the presence of microplastics and the pollution risk of microplastics in some bivalve organisms in the South Central Coast – Vietnam.

2. Research Objects and Methods

2.1. Sampling in the South Central Coast - Vietnam

Nine sampling sites (from VT1, VT2… to VT9) were collected with bivalves of rock oysters *Saccostrea sp*, and sea clams *Venus sp* in the south central coast for microplastic analysis in these tissues (Figure 1). All samples were collected in June, 2023.

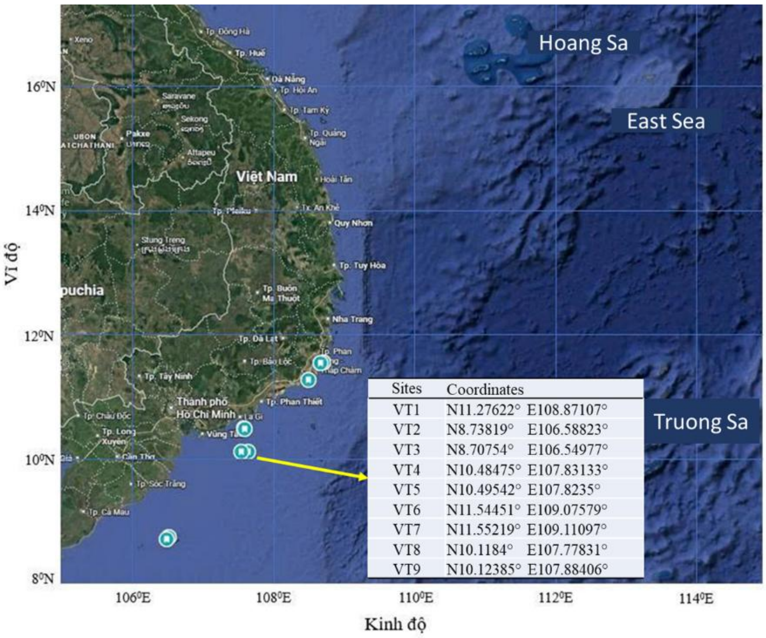

Figure 1. Sampling sites in the studied area.

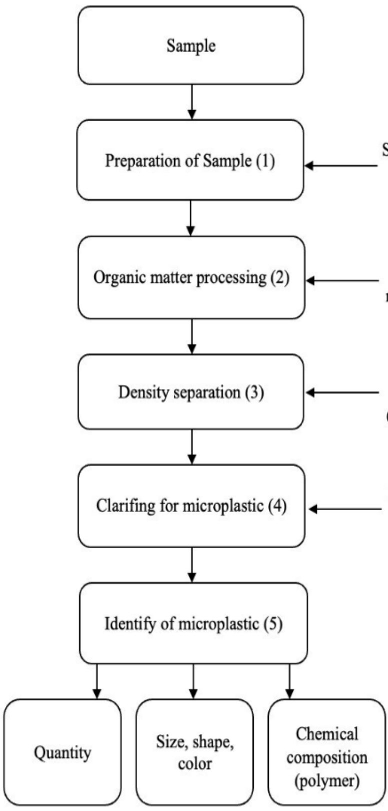

Figure 2. Diagram of MPs analysis.

2.2. Analytical Methods in the Laboratory

Bivalve samples were collected and brought to the laboratory and stored in the refrigerator. Before analysis, the samples were taken out to cool naturally. The microplastic analysis process is modified and presented in the diagram below [14] (Figure 2).

2.3. Microplastic Analysis Method by Using Micro Fourier Infrared Spectroscopy (Micro-FTIR) Equipment

Samples are analyzed by using a Micro-FTIR infrared microscope device (Nicolet iN10MX), Thermo Scientific - USA, allowing analysis and identification of microplastics in samples. The steps are as follows:

Step 1: Spreading the sample on a flat sheet with a reflective surface;

Step 2: Putting the flat plate into the LDIR. The software automatically start the analysis; Step 3: The device uses single waves to automatically scan the surface of the flat plate containing the sample and determine the location of the particles. Next the device isolates the identified particles (appearing as bright spots) and collects the IR spectrum of each particle; Step 4: Each spectrum is then compared with a spectral library to determine the chemical composition of each particle and polymers were determined by the softwave OMNIC Picta.

The data obtained such as total amount of microplastics in the sample; size (in µm) of microplastics; shape (fragment, round, oval...); and polymer composition in microplastics.

Calculation methods

Microplastic density in researched bivalve samples is expressed as the number of microplastics per gram of weight or the number of microplastics per individual according to formulas (1) and (2):

$$C_w = MPs_i/W_i \text{ (particles/gram)} \quad (1)$$

$$C_{in} = MPs_i/n_i \text{ (particles/individual)} \quad (2)$$

MPs_i: the quantity of microplastics of each individual organism analyzed (microplastics);

n_i: the number of individuals at each sampling site (microplastics);

W_i: the wet tissue weight of individual oysters at each sampling site (g);

C_w: microplastic density (particles/gram);

C_{in}: microplastic density (particles/ individual).

Calculating the percentage according to size distribution, shape and polymer chemical composition of microplastics in each analyzed sample as formula (3):

$$T\% = MP_i/\sum MP \quad (3)$$

MP_i: the number of microplastics distributed according to each size, shape, and chemical composition of microplastics in each research sample (particles);

$\sum MP$: total number of microplastics in each analyzed sample (particles).

2.4. Risk Assessment Method of Microplastics

The PHI (polymer hazard index) is used to assess health risks from microplastics based on Lithner et al., 2011. The PHI risk index is calculated according to the following formula with identified polymers in sample having the risk score [6]:

$$PHI = \sum P_n S_n$$

P_n: The average percentage of each microplastic polymer in all samples (%);

S_n: The risk score of each type of microplastic.

PHI ≤ 100 indicates a low risk level of microplastic pollution and conversely PHI > 100 represents a high risk level of microplastic pollution to human health.

3. Results and Discussion

3.1. Density of Microplastics in Bivalves in the South Central Coast

The density of MPs in bivalve samples in the South Central region of some natural rock oyster species *Saccostrea* sp. (VT1 – VT7) and sea clam *Venus* sp. (VT8 – VT9) is illustrated in

Table 1. The results showed that microplastics were detected in all bivalve samples, with densities ranging from 0.25 - 1.59 particles/g-fresh tissue weight. In which, the highest density of microplastics is at location VT3 with 1.59 particles/g-fresh tissue weight and the lowest at location VT5 (0.18 particles/g-fresh tissue weight).

Compared with the research about microplastic pollution in the bivalves mollusks in Danang, Vietnam of Do Van Manh et al, 2022, microplastics were identified and quantified in farmed Pacific oysters (*Crassostrea gigas*). The results showed that the average MP density in oysters was about 2.36 ± 2.14 particles/g (wet weight) and 33.25 ± 25.93 particles/individual [12]. Those microplastic density results were higher than the microplastic densities of bivalve species *of Saccostrea sp* (VT1 – VT7) and sea clam *Venus sp* (VT8 – VT9) collected from 9 sampling sites in the present study. These results explained that bivalves in the sampling sites were natural and they lived at the bottom of the sea where is cleaner and it was far from the farming area; thus, microplastics in these sampling sites were less than those in previous studies.

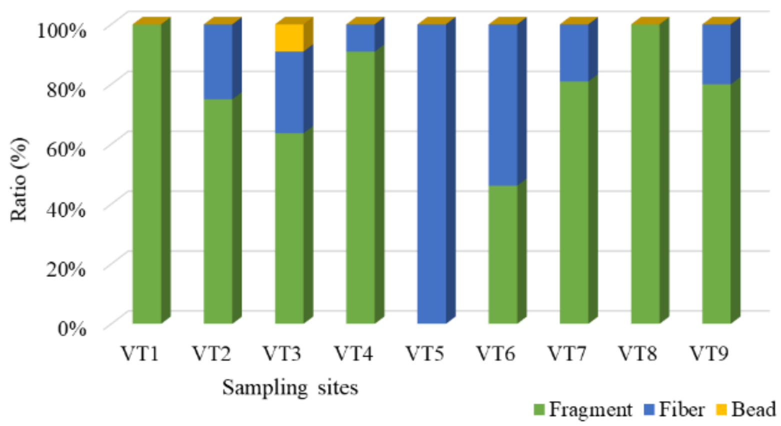

Figure 3. The percentage of microplastic shapes in bivalve samples at 9 survey points.

Table 1. Results of microplastic density in some species of rock oysters and natural sea clams at the survey sites (n= 6)

Sites	Sample sites	Fresh tissue weight (g)	Microplastic density (particles/individual)	Microplastic density (particles/g-fresh tissue weight)
Bai Can Breda (Breda bank)	VT1	2.58 ± 0.36	1 ± 0.5	0.39 ± 0.12
Hon tre nho	VT2	8.96 ± 0.56	8 ± 1.2	0.90 ± 0.28
Hon tre lon	VT3	6.92 ± 0.38	11 ± 1.5	1.59 ± 0.59
De Britto 1	VT4	13.56 ± 0.58	11 ± 0.8	0.81 ± 0.38
De Britto 2	VT5	16.26 ± 0.69	3 ± 0.56	0.18 ± 0.05
De Chateaurenault	VT6	9.63 ± 0.37	13 ± 1.86	1.35 ± 0.45
Pateau De Corail	VT7	16.26 ± 0.72	21 ± 3.26	1.29 ± 0.46
De L'Astrolabe 1	VT8	16.06 ± 0. 86	4 ± 0.86	0.25 ± 0.11
De L'Astrolabe 2	VT9	9.13 ± 0.42	5 ± 1.15	0.55 ± 0.52

3.2. Shape and Size of Microplastics in Some Bivalves in the South Central Coast

In this study, a total of 77 microplastic particles were found inbivalve samples of 9 sample sites in the South Central Coast. The largest number of microplastic shapes is fragment found up to 55 particles, accounting for more than 71%. Next, fiber shape was 21 particles, accounting for about 27%. Finally, the form of bead had 1 particle about 1% in total. At the sampling locations, the percentage of microplastic shapes was depicted in Figure 3. Generally, at almost all sampling sites (up to 8/9 sampling sites), the percentage of fragments was the majority, with about over 50%, followed by fibers and finally bead.

The results of this study are similar to the study of MP size in Pacific oysters (*Crassostrea gigas*) raised in Da Nang Bay, Vietnam. The most common microplastic shape is fragment, accounting for 79.32%, followed by fibers, accounting for 20.30% [12]. Meanwhile, in Ha Long Bay, 3 polymers were collected, including shapes of fragment, fiber, and bead with corresponding rates of 69.20%, 23.55% and 7.25%, respectively, at the Bai Chay and 72.43 %, 22.63 % and 4.94 %, respectively at Gieng Day.

The size of microplastics in some natural rock oyster species *Saccostrea sp* (VT1 –VT7) and sea clam *Venus sp* (VT8 -VT9) at sampling sites is shown in Figure 4.

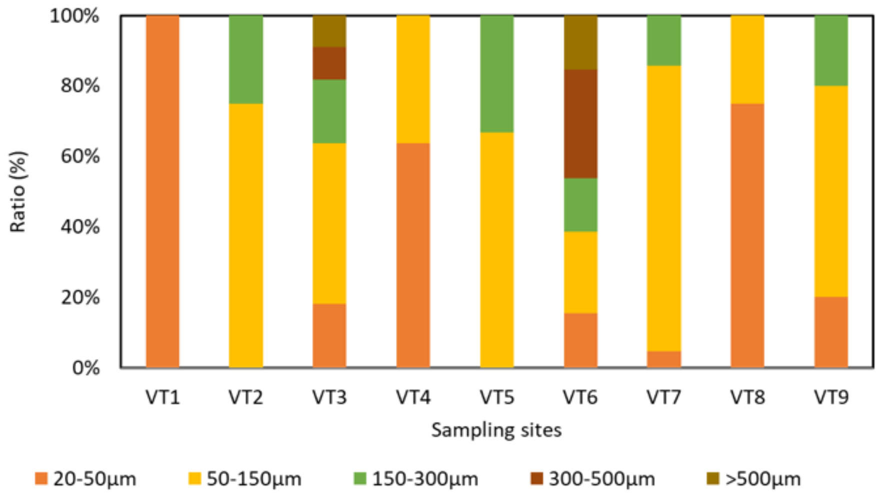

Figure 4. Size of microplastics in bivalve samples.

The size of MPs was divided into 5 groups of different sizes 20 – 50 µm, 50 – 150 µm, 150 – 300 µm, 300 – 500 µm and > 500 µm) (Figure 4). The results showed that almost all MPs have sizes ranging from 50 - 300 µm. MPs with size from 50 - 150 µm were optimal, accounting for 41/77 of the total detected MPs (about 53%). Next, MPs with sizes from 150 - 300 µm, accounting for 14%. MPs with sizes over 500 µm account for the lowest proportion (3.9%) of the total MPs and only appear at 2 sampling sites (VT3 - VT6). MPs with sizes from 20 – 50 µm appeared in all sampling locations but in a small proportion (22%).

Compared with the research on microplastic pollution in the bodies of bivalve mollusks by Nguyen Duy Thanh et al., 2021, at two survey sites in Ha Long Bay. Microplastic sizes smaller than 150 µm were dominant, accounting for 81.88% and 86.83% of the total mussel samples at Bai Chay and Gieng Day, respectively. This study's survey results were higher than those collected from 9 sampling sites (about 10%).

3.3. Microplastic Polymers in Bivalves of the South Central Coast

Figure 5 illustrates the composition of polymers detected in bivalve samples along the South Central Sea that were measured by Micro - FTIR Nicolet iN10MX infrared microscope.

The results showed that there were 10 types of microplastic polymers identified in bivalves of some natural rock oyster species *Saccostrea sp* (VT1-VT7) and sea clam *Venus sp* (VT8-VT9) in the South Central. They were polymers such as Polyethylene Terephthalate (PET), Nylon, Polyethylene (PE); high density; average MW ca, HDPE 30060M, Melamine-urea-formaldehyde resin, Cellophane, Polyester, Teflon, Low Density Polyethylene Em 460. The percentage of detected polymers was shown in Fig. 5. Among them, the number of PET particles was predominated (58%).

Next, nylon were detected 8 particles, accounting for 10%. These are polymers produced in popular plastic products in daily household items and industrial plastic materials. Meanwhile, other types of microplastics account for an insignificant amount of 1 - 9%. In the fact that, PET is used to produce water bottles, nylon in book bag and furniture packaging materials. Therefore, it was proposed that these PET materials were accumulated mainly for a long time in the environment and it maybe contaminated into the bivalves in this sampling sites.

PE is a material used to produce clothes, plastic covers of electronic devices, and tires. Phenol resin (PF) is known as the first commercial resin. They are used to produce molded products such as billiard balls, laboratory countertops, and as coatings and adhesives. The presence of PE suggests that the microplastics were found with origin in abundance from items on land. Thus, the results truly reflect the proportion of types and quantities of polymers found, reflecting the environmental status of the sampling sites [9, 15].

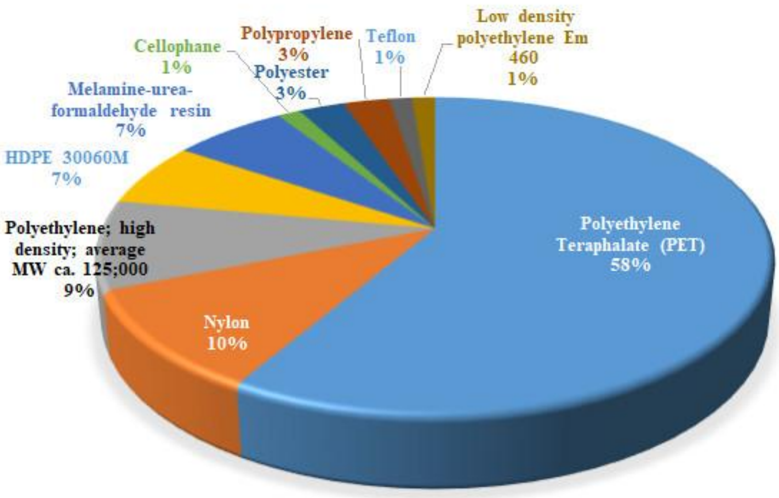

Figure 5. Composition of microplastic polymers in bivalves of the South Central Coast.

3.4. Risk of Microplastic Pollution in some Bivalves in the South Central region

Calculation of the risk level was based on the percentage of each type of polymer detected and the corresponding risk score for the polymer types presented in the survey sample. It showed that the risk level of microplastics in some bivalves in the South Central region was high with a PHI index of 100.81. Thus, at this level, bivalves are generally affected by microplastic pollution and PHI > 100 as prescribed, is an indicator of high risk level of microplastic pollution. Research results have shown that bivalves such as some natural rock oyster species *Saccostrea sp* (VT1 – VT7) and sea clam *Venus sp* (VT8 –VT9) here are unsafe and unsuitable for human consumption. In the fact that, these natural bivalves lived in the bottom of the sea and sampling were difficult and these bivalves were not also used as sea food here due to the shapes of bivalves were weird and different from them in the farming area. Therefore, in this study, it became a warning for human beings to avoid using the natural bivalves in the sampling sites as sea foods.

4. Conclusion

The initial study provided acknowledge of MP pollution in this survey area. The results showed that MPs appeared in all analyzed samples. The average concentration of microplastics in 9 sampling sites in the South Central Coast ranges from 1 to 21 particles/individual and from 0.25 to 1.59 particles/g- fresh tissue weight. The detected microplastic size was mainly smaller than 150 µm. The polymer types of MPs in the studied bivalve samples were determined to be very diverse, with 10 types of MP polymers detected, PET was the most common polymer with 58%. These results provide useful data in the management of MP pollution as well as potential ecological risk assessment with PHI value of 100.81 as prescribed, is an indicator of high risk level of microplastic pollution. However, this is only preliminary study, so more in-depth studies need to be conducted to evaluate the conditions and factors that can affect the distribution and characteristics of MPs and their effects for the environment, marine life, and human health in the future.

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