TẠP CHÍ KHOA HỌC
TRƯỜNG ĐẠI HỌC QUY NHƠN

Mật độ và đặc điểm của vi nhựa ở ngao Bộp (*Mactra grandis*) phân bố ở đầm Cù Mông, tỉnh Phú Yên

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TÓM TẮT

Mục tiêu của nghiên cứu này là đánh giá ô nhiễm vi nhựa ở ngao Bộp sinh sống ở đầm Cù Mông, tỉnh Phú Yên. Ngao Bộp thu được ở đầm được xử lý bằng KOH 10% để xác định các loại vi nhựa tồn tại trong ống tiêu hóa. Kết quả cho thấy có hai dạng vi nhựa trong ống tiêu hóa của ngao Bộp là vi nhựa dạng sợi và vi nhựa dạng mảnh, với tổng mật độ trung bình là 1,53 vi nhựa/cá thể ở mùa nắng và 8,93 vi nhựa/cá thể ở mùa mưa. Chiều dài các sợi vi nhựa chủ yếu nằm trong khoảng 300 – 2500 µm trong khi diện tích các mảnh vi nhựa nằm trong khoảng 45.000 - 600.000 μm^2. Màu sắc vi nhựa dạng sợi đa dạng hơn so với dạng mảnh, tuy nhiên nhìn chung màu trắng và màu vàng là hai màu chiếm ưu thế của các mẫu vi nhựa, ngoài ra còn có màu xanh lá, tím và xanh biển.

Từ khóa: *Ngao Bộp, ống tiêu hóa, vi nhựa, đầm Cù Mông, ô nhiễm.*

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Concentration and charateristics of microplastic in Big brown mactra clam (*Mactra grandis*) distributed in Cu Mong lagoon, Phu Yen province

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ABSTRACT

The objective of this study is to assess microplastic contamination in Big brown mactra clam inhabiting in Cu Mong lagoon, Phu Yen province. The individuals of this clam collected from the lagoon were treated in KOH 10% to determine microplastic particles in their digestive system. The results disclosed two shapes of microplastic in the digestive system of Big brown mactra clam called fibers and fragments with average microplastic concentration of 1.53 particles per individual in sunny season and 8.93 ones per individual in rainy season. The length of the fibers was mostly from 300 to 2500 µm while the area of the fragments was dominant in the range of 45.000 to 600.000 μm^2. The colour of fibers was more diverse than that of fragments; however, in general, white and yellow were the predominant colours of the microplastic particles, followed by green, purple and blue.

Keywords: *Big brown mactra clam, digestive tract, microplastic, Cu Mong lagoon, contamination.*

1. INTRODUCTION

Microplastic pollution is a globally concerned problem. Microplastics, especially synthetic fibers and artificial fibers, have been found in many water bodies such as lakes, rivers, estuaries, seas, etc. These microplastics can be transported through food webs in freshwater, saltwater environments and can affect human health.[1-3] Because of such hazards from microplastics, a lot of researches on microplastics have been done in many different areas in the world. Lot of researchers have conducted many researches on microplastic in the environments. For example, Free et al.[4] did the microplastic research in Hovsgol lake – Mongolia; Cabernard et al.[5] compared Raman and Fourier transform infrared spectroscopy to quantify microplastics in the aquatic environment; Wenfeng aet al.[6] carried out the research of microplastic in the domestic freshwater in China; Zhao et al.[7] studied on microplastic in surface water in Yangtze river system – China. In addition, studies on microplastic accumulation in molluscs have been considered and done by lots of researchers such as Li et al.[8] for *Mytilus edulis* in coastal areas – China, with a density of 1.5 – 7.6 particles/individual; Abolfazl et al.[9] for *Amiantis umbonella, Amiantis purpuratus, Pinctada radiate, Cerithidea cingulata, Thais mutabilis* in coastal areas of Persia bay – Iran, with density of 3.9 – 6.9 particles/individual; Li et al.[10] for 9 bivalve species as *Sc. subcrenata, T. granosa,*

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My. Galloprovincialis, P. yessoensis, A. plicatula, Si. Constricta, R. philippinarum, Me. lusoria, C. sinensis collected from seafood markets in China, with 4.3 – 57.2 particles/individual; Sami et al.[11] for 6 species as *Mytilus galloprovincialis, Ruditapes decussatus, Crassostrea gigas, Hexaplex trunculus, Bolinus brandaris, Sepia officinalis* in Bizerte lagoon –Tunisia, with a density of 703.95 ± 109.80 to 1482.82 ± 19.20 particles/kg fresh body weight. However, although Vietnam is the fourth largest plastic emitting country in the world,[12] so far, researches on microplastic has been rare. It is noted that there are a few studies on the accumulation of microplastics in organisms carried out by some researchers such as the study of Nam et al.[13] on green mussels in Tinh Gia - Thanh Hoa.

Phu Yen as well as many other provinces in Vietnam is being seriously affected by plastic waste. In particular, this is one of the provinces with a border adjacent to the sea, a long coastline, and terrain sloping from West to East, so most of plastic wastes discharged eventually drift to the coastal areas such as bays, lagoons and then flow to seas and oceans. However, there has been no study on microplastic carried out in Phu Yen to assess the pollution level of this waste.

Cu Mong, the habitats of many valuable seafood species as seahorse, oyster, Big brown mactra clam, is one of two important lagoons of Phu Yen, that brings to benefits for local residents. Big brown mactra clam is one of the molluscs widely distributed in this lagoon and is a favorite seafood species of local residents well as widely consumed in other provinces. Based on the field surveys, it can be seen that Big brown mactra clam is a benthic species and often bury itself in the bottom. Like many other bivalve molluscs, Big brown mactra clam is the filter feeder with feeding mainly on algae and organic detritus. With such feeding habits, this species can eat microplastic particles together with their natural food. However, Arapov et al.[14] suggested that although bivalve species are filter feeders, they are able to select food basing on size, shape, nutritional value or chemical composition on the surface of food particles. Therefore, in this study, we chose Big brown mactra clam to study to see whether this species is contaminated microplastics in the digestive tract.

2. METHODS

2.1. Sample collection

The clams were collected from fishermen fishing in the range of V1 ($13^0$35ˀ59"N - $109^0$13ˀ54"E) and V2 ($13^0$32ˀ52"N - $109^0$16ˀ26"E (Figure 1), then were put into the zip bags, frozen and brought to the lab for later analysis.

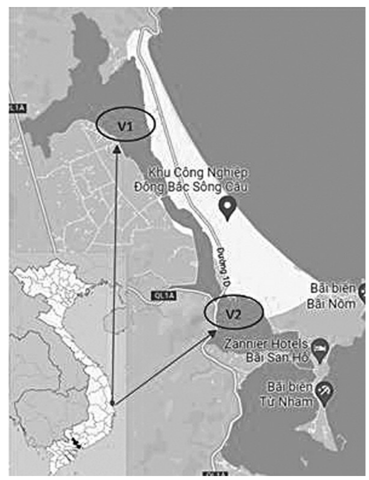

Figure 1. The sampling sites in Cu Mong lagoon

Samples were collected every three months during 3 months of wet season (October to December) and 3 months of dry season (March to May). A total of 60 individuals of clams were collected (10 clams/month) to examine.

2.2. Sample treatment and analysis

Each individual of clam was separately treated and analysed. At the lab, clams were rinsed by water filtered through the glass fiber filters (GF/A, pore sizes of 1.6 μm), then weighed (weight of shell and muscle).

Next, the clams were put on petri disk to dissect and got the digestive tracts.

The digestive tracts were contained in glass beakers and treated in KOH 10% at 60 ^{0}C within 24 hours according to Alexandre.[15]

The samples after treated in KOH were filtered through 1mm mesh size sieve to discharge extraneous matters and collect microplastic particles from 1- 5 mm.

The samples filtered through the 1 mm sieve were filtered through 250 µm mesh size sieve, then the samples on the sieve were rinsed into a beaker to do the overflow technique using saturated NaCl solution. Finally, sample solution was filtered through a 1.6 µm GF/A filters according to the method of Emilie et al.[16]

The filters were observed under the Leica S9i stereomicroscope to determine the shape of microplastics based on the classification of Free et al.,[4] that includes 5 shapes such as fibers, fragments), pellet, film and foam. The LASX software of stereomicroscope was used to take pictures, measure the size and determine color of each microplastic. Then, all details of analysis were saved as Excel format for later data analysis.

2.3. Data analysis

Data for analysis include shape, concentration, size (length of fibers and area of fragments) and color of microplastic. Because of lack of facilities to determine the chemical origin of microplastics, we only considered fibers from 300 – 5000 µm in length and fragments from 45,000 – 25,000,000 μm^2 in area according to suggestion of GESAMP.[17]

Microsoft Excel 2013 was used to calculate the necessary parameters and make diagrams. Anova single factor in Microsoft Excel 2013 was applied to check the concentration difference of microplastic.

2.4. Microplastic contamination control

To control microplastic contamination from the surrounding environment during sample treatment and analysis, we followed suggestions of GESAMP[17] such as cleaning the working area with alcohol before analysing and treating samples, wearing cotton clothes and rubber gloves, rinsing equipment with water filtered through GF/A glass fiber filter (pore size 1.6 µm) before use. In addition, at each period of sample analysis and treatment, we placed a new filter paper in a petri dish nearby places we were working to check microplastic contamination. After finishing each process, we observed this filter paper under the Leica S9i stereo microscope to check microplastic contamination from the surrounding environment.

During sample analysis and processing, we did not detect any microplastics on the control filter papers.

3. RESULTS AND DISCUSSION

3.1. Microplastic concentration

Two shapes of microplastic found in the digestive tracts of Big brown mactra clams were fibers and fragments. The results in Table 1 showed that in the same season, concentration of fibers was higher than that of gragments. The study results of Li et al.[8] on *Mytilus edulis*, Li et al.[10] on 8 mollusc species (*Sc. subcrenata, T. granosa, My. galloprovincialis, P. yessoensis, Si. constricta, R. philippinarum, Me. lusoria, C. sinensis*) and Sami et al.[11] on 6 mollusc species (*Mytilus galloprovincialis, Ruditapes decussatus, Crassostrea gigas, Hexaplex trunculus, Bolinus brandaris, Sepia officinalis*) also indicated the dominance of fibers compared to fragments.

For different seasons, microplastic concentration (fibers, fragments and total) in wet season was higher than that in dry season ($p < 0,05$). It can be seen that weight of clams was not significantly different between two seasons. The results also indicated that the correlation between weight of clams and number of microplastic in wet season was very low ($r = 0.03$) while that in dry season was negative ($r = -0.55$). Therefore, it can be speculated that the difference of microplastic concentration in the digestive tract of clams in two seasons may be due to other factors instead of clam size. Clearly, there is no river going to Cu Mong lagoon and water in the lagoon is exchanged with the sea through an unique gate. Thus, in rainy season, wastes including plastic waste are washed away from the surrounding areas into the lagoon, but because there is no strong flows from the river, microplastics are easily deposited in the lagoon instead of being washed into the sea. In contrast, in the dry season, the rainfall is low, so there may be a small amount of waste around the lagoon going into the lagoon and hence the amount of microplastics in the dry season will be less than that in rainy season. Therefore, with bottom living habit and filter feeding, Big brown mactra clams can ingest a higher number of microplastic in rainy season compared to dry season.

Table 1. Microplastic concentration in the digestive tracts of Big brown mactra clams

Season	Average weight of clams (g)	Number of microplastic fibers per individual	Number of microplastic fragments per individual	Total number of microplastic per individual	Correlation coefficient between clam weight and number of microplastic (r)
Dry season (n=30)	14.97 ± 1.28^a	0.87 ± 0.53^b	0.67 ± 0.27^b	1.53 ± 0.64^b	-0.55
Wet season (n=30)	12.80 ± 4.77^a	7.30 ± 3.28^a	1.63 ± 1.54^a	8.93 ± 3.72^a	0.03

Remark: For each parameters (each column), the different letters indicate the significant difference ($p < 0.05$)

Some researchers also present microplastic contamination level as number of microplastic particles per individual such as Li et al.,[8] Abolfazl et al.[9] Although these authors examined the microplastic with size much smaller than this in our study, microplastic concentration in those studies just fluctuated from 1.5 to 7.6 particles/individual while this number in our study was from 1.53 to 8.93 particles/individual (Table 2). This indicates that microplastic contamination level in Cu Mong lagoon is pretty high, which is a bad sign of environment for this ecosystem.

Table 2. Microplastic contamination in some mollusc species

Authors	Study sites/regions	Studied species	Microplastic concentration	Microplastic size (µm)
Li et al., 2016[8]	Coastal areas in China	*Mytilus edulis*	1.5 – 7.6 (particles per individual)	5 – 5000
Abolfazl et al., 2018[9]	Coastal areas in Persia bay, Iran	*Amiantis umbonella, Amiantis purpuratus, Pinctada radiate, Cerithidea cingulata Thais mutabilis*	3.9 – 6.9 (particles per individual)	10 – 5000
This study	Cu Mong lagoon, Phu Yen, Vietnam	*Mactra grandis*	1.53 – 8.93 (particles per individual)	300 - 5000

3.2. Microplastic size

Microplastic fibers found in this study had the different ranges of size but there was difference between wet season and dry season. In the wet season, microplastics almost existed in all size groups of 300 - 5000 µm (except the size group of 4500 - 4700 µm) while in the dry season, microplastics were only in the size group of 500 - 3100 µm (Figure 2).

In general, the microplastic fibers were mainly from 300 - 2500 µm in length, but there was different distribution between dry season and wet season. Specifically, in wet season, the size classes of 1300 - 1500, 1500 - 1700 µm and 1700 - 1900 µm had the largest number, accounting for 10.68%, 9.26% and 10.19% of the total, respectively while the size classes of 1500 - 1700, 1700 - 1900, 1900 - 2100 and 2100 - 2300 µm accounted for the highest proportion in the dry season, with 11.54%, 19.23%, 15.38% and 11,54% of the total respectively.

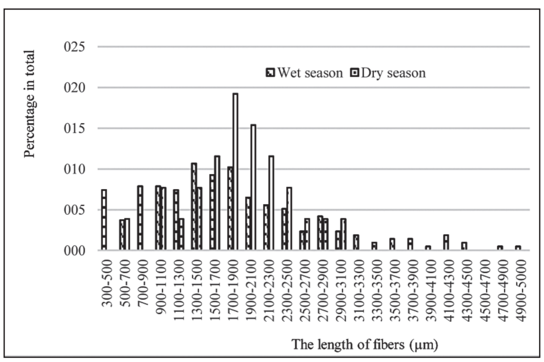

Figure 2. The length of fibers (µm) according to occurrence rate (%)

The size distribution of microplastic fragments also differed between the two seasons. In the dry season, fragments were mostly in the size classes of 200,000 - 400,000 µm^2 and 400,000 - 600,000 µm^2 (accounting for 45.00% and 25.00% of the total, respectively) and there was no fragments in the size group 800,000 - 1,000,000 µm^2. In wet season, there was no microplastic in the size group of 1,000,000 - 1,200,000 µm^2 and mainly in the size group of 45,000 - 200,000 m^2 and 200,000 - 400,000 µm^2, with 47.92% and 27.08% of the total, respectively (Figure 3).

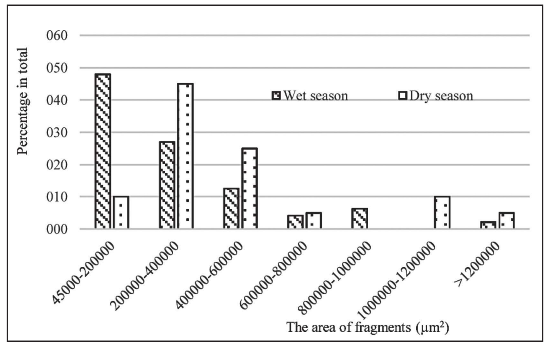

Figure 3. The area of fragments (µm^2) according to occurrence rate (%)

In general, our study results show that the small size class is dominant in total number of microplastic found. Specifically, most of fibers have the length of 300 - 2500 µm, accounting for 88.46% in total in dry season and 83.59% in total in wet season; fragments are predominant in area class of 45,000 – 600,000 µm^2, with 80% in total in dry season and 86.5% in total in dry season (Figure 4). Cabernard et al.,[5] Wenfeng et al.,[6] Zhao et al.[7] also indicated the dominance of small size microplastics. Tayler et al. reported that water currents and UV radiation are responsible for producing a large number of small size microplastics.[18]

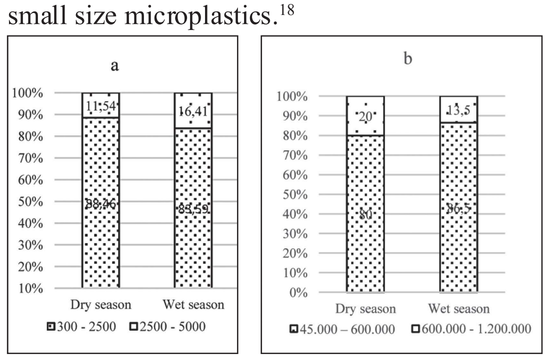

Figure 4. The pooled percentage of microplastic according to length (µm) of fibers (a) and area (µm^2) of fragments (b)

3.3. Colors of microplastics

The colors of microplastics found in the digestive tracts of Big brown mactra clams were quite diverse. Of which, the colors of microplastic fibers were more diverse than this of microplastic fragments. Specifically, the fibers had 10 colors (blue, black, brown, green, orange, pink, purple, white, yellow, gray) while the fragments only

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comprised 5 colors (white, purple, yellow, orange, green) (Figure 5 and Figure 6).

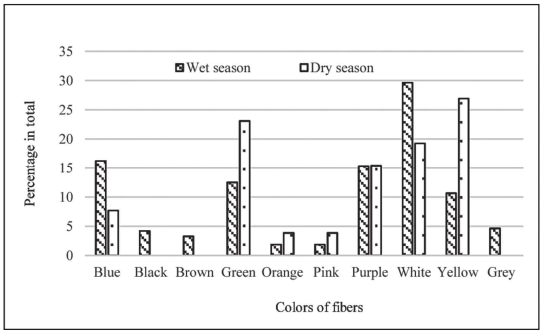

Figure 5. The colors of microplastic fibers according to occurrence rate (%)

The color of fibers changed between the seasons. In dry season, yellow predominated (26.92%), followed by green (23.08%), white (19.23%), and purple (15.38%) while white dominated (29.63%), followed by blue (16.20%), purple (15.28%) and green (12.50%) in rainy season.

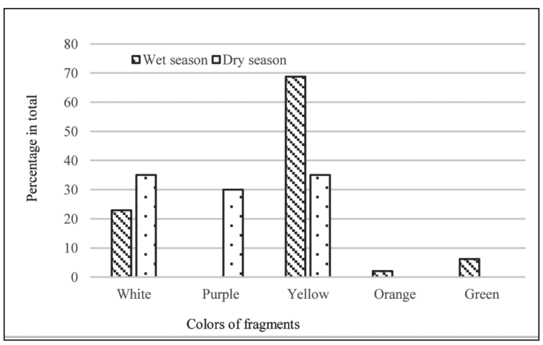

Figure 6. The colors of microplastic fragments according to occurrence rate (%)

For fragments, white and yellow were the main colors, and purple also contributed a large proportion in total. However, there is a difference between two seasons. In wet season, the fragments had 4 colors, of which yellow accounted for the highest percentage (68.75%), followed by white (22.92%) while there were only 3 colors, that are yellow, purple and white with the approximately equal proportions (Figure 6).

In general, in this study, white and yellow are the predominant colors, in addition to green, blue and purple. Ayu et al.[19] reported that white is also the dominant color found in the digestive tracts of fish (accounting for 79.2%) but followed by blue (7.03%) instead of yellow as in our study. In another study on six mollusc species (*Mytilus galloprovincialis, Ruditapes decussatus, Crassostrea gigas, Hexaplex trunculus, Bolinus brandaris and Sepia officinalis*), black, blue, and white are the dominant colors for microplastic fibers and blue, red and black are the main colors of fragments.[11] Thus, the colors of microplastics found in aquatic animals are quite different, which might be due to the different sources of microplastics in the study locations. This is also reported by Gallagher et al.[20]

4. CONCLUSION

Microplastics accumulate in the digestive tracts of Big brown mactra clams distributed in Cu Mong lagoon, Phu Yen province with the concentration of 1.53 to 8.93 particles/individual. The clams collected in the wet season are contaminated with higher number of microplastics than those collected in dry season.

Microplastic fibers as well as microplastic fragments found in the digestive tracts of this clam species are mostly in the small size class (300 - 2500 μm for fibers and 45,000 - 600,000 μm^2 for fragments).

The color of microplastics in the digestive tracts of the clams is quite diverse, in which the color of fibers is more diverse than this of fragments. In addition, the color of microplastics also changes in two seasons.

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