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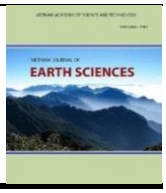

Contamination of microplastic in bivalve: first evaluation in Vietnam

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ABSTRACT

Microplastics (MP) are omnipresent in ecosystems. Some studies focus on MP fates and their toxicology on biota for the last ten years in the world. In the present study, MP was identified in bivalve (Perna Viridis) collected in Vietnam for the first time using micro-Fourier transform infrared Microspectroscopy (μFTIR) technique. Organisms were digested by KOH 10% solution then separated using KI 50% solution. The average concentration evaluated at 2.60 MP/individual and 0.29 MP/gram of wet tissue. Six types of MP were found with a high proportion of polypropylene (PP) (31%) and polyester (23%). MP characterizations were also observed which bring to much important information such as the source of MP contamination in bivalve from Vietnam. Nevertheless, more work needs to be invested in the future such as on different species or environment compartments which permit to the global view of MP contamination in Vietnam.

Keywords: Microplastic contamination; bivalve; *Perna viridis*; μFTIR, Vietnam.

1. Introduction

Worldwide production of plastic was estimated up to 348 million tons in 2017 (PlasticsEurope, 2018) which could be explained by many advantages of plastics such as inexpensiveness, water, and corrosion resistance, chemically inertness, easily molding, and they exhibit good thermal and electrical insulating properties. However, half of them was thrown after only one used (Hopewell et al., 2009). About 5 to 12 million tons of plastics are discharged to oceans each year (Jambeck et al., 2015). Consequently, many reported for the ingestion and entanglement of plastics by marine animals ranging from zooplankton to mammals (Gall and Thompson, 2015; Thompson et al., 2009). Microplastics (MP) was defined as plastic particles between 1 μm to 5 mm in size by Arthur et al. (2009) and accepted by most of the studies. There are two sources of MP in

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the environment. Primary MP used as an additive in toothpaste, cosmetics for examples while secondary MP as the result of the fragmentation of plastic litter in the environment under impacts of many factors as erosion, abrasion, photooxidation, temperature, degradation. They accumulate in the environment compartments which bring to many influences on biota. Numerous studies have been investigated to determine the quantities of MP in the different environmental compartments such as seawaters (Doyle et al., 2011; Eriksen et al., 2013; Lucia et al., 2014) or sediment (Carson et al., 2011; Phuong et al., 2018c) and bivalves (Phuong et al., 2018a; 2018b) in the world. Because of the feeding mode, bivalves are known as bio-indicator for evaluation of MP contamination on their environment matrix. They were studied in different countries such as China, French, England, and Belgium. These studies demonstrated that bivalves were contaminated by different MP (type, concentration, form). However, the different results could be obtained when using different protocols concerning chemical agents for digestion steps or the identification technic. None of the studies was found in Vietnam to this moment for evaluating MP in bivalves. Hence, the main goal of this paper is the first time for evaluating MP contamination in an Asian green mussel (*Perna viridis*) sampled in Vietnam.

2. Materials and methods

In the present study, the Asian green mussel (*P. viridis*) were selected for monitoring MP contaminant levels in coastal waters due to their wide distribution, easy sampling and they are recognized as commercially valuable seafood in Vietnam. *P. viridis* can be found to be abundant in the center zone of Vietnam (Nguyen et al., 1996). Considering the above mentioned, mussels in adult size (shell length more than 8 cm) were sampled in a brackish water zone in Tinh Gia, Thanh Hoa province in March 2019. They are represented for wild mussel's type. Samples were conserved with aluminum foil in the refrigerator until analysis. This conservation evitable of MP contamination than the use of plastics materials. Cross-contamination in the laboratory was evitable with coat lab, the nitrile gloves were worn at all time and all sample processing was performed in a clean laminar flow cabinet. All equipment was rinsed with water ultra-pure before of utilization.

The KOH and KI pellets using for digestion step were purchased from Sigma-Aldrich. Cellulose nitrate filter from the Whatman, Germany (12 µm in porosity and 47 mm in diameter). Identification of MP was performed by using of the technic of µFTIR (Spotlight 200i FT-IR microscopy system, PerkinElmer).

Byssus of bivalves was eliminated before analysis. Masse of tissue and length shell were noted individually. The analysis protocol including four steps as sample preparation, sedimentation, filtration, and identification was demonstrated in Phuong et al. (2018a). In brief, five mussels were analyzed individually. Each sample was performed with 50 mL of KOH 10% solution. After of digestion step, the solution was separated two folds with KI 50% solution using a separating funnel. Cellulose filter was used for the filtration step. Filters were then dried in ambient temperature and closed in glass Petri until analysis. Particles rested on the filter were observed and identified directly on the µFTIR machine. Each spectrum was compared with the standard polymers database. One item was accepted as MP when research score superior to 60%.

MP concentration was calculated by the number of MP on masse of tissue (MP/g) and by number of MP on mussel (MP/individual). Average of concentration calculated from fives replicates using Excel software.

3. Results and discussions

3.1. MP Concentration in bivalve

The blank analysis of this protocol was detail described in Phuong et al., (2018a, 2018b). None of the MP particles was found on the blank filter. It seems to assurance that cross-contamination during the procedure by MP is evitable.

MP abundance in bivalve was about 0.29 (±0.14) MP per gram of wet weight soft tissues and 2.60 (±1.14) MP per individual (N = 5). An example of polypropylene (PP) MP and the spectrum are presented in Figs. 1, 2.

In order to have a view more details on MP contamination in bivalves, table 1 presented the results from different works found in literature.

As shown in table 1, MP contamination ranging from 0.1 to 20 MP/g of wet tissue. High difference between the results was reported. Evidently, it could be from the sampling sites with different MP pollution levels. Our result showed MP concentration in bivalve in Vietnam was higher than in of the ones in Europe (Phuong et al., 2018b; Vandermeersch et al., 2015) but lower than bivalves from of China (Li et al., 2016) and Canada (Mathalon and Hill, 2014). By the same species, MP concentration in mussel from Vietnam is very lower than this one from of China and Indonesia (Khoironi and Anggoro, 2018; Qu et al., 2018). Although, the difference of the results could be from by the protocols utilized (Phuong et al., 2018a). Especially, the identification technic has played a role important of MP found in bivalve. While the observation seems to not be enough to identify of MP nature, the Raman spectroscopy is predominant with small MP (down to 1 µm in size) and the µFTIR identifiable of MP ranging 3 to 800 µm depending on working mode.

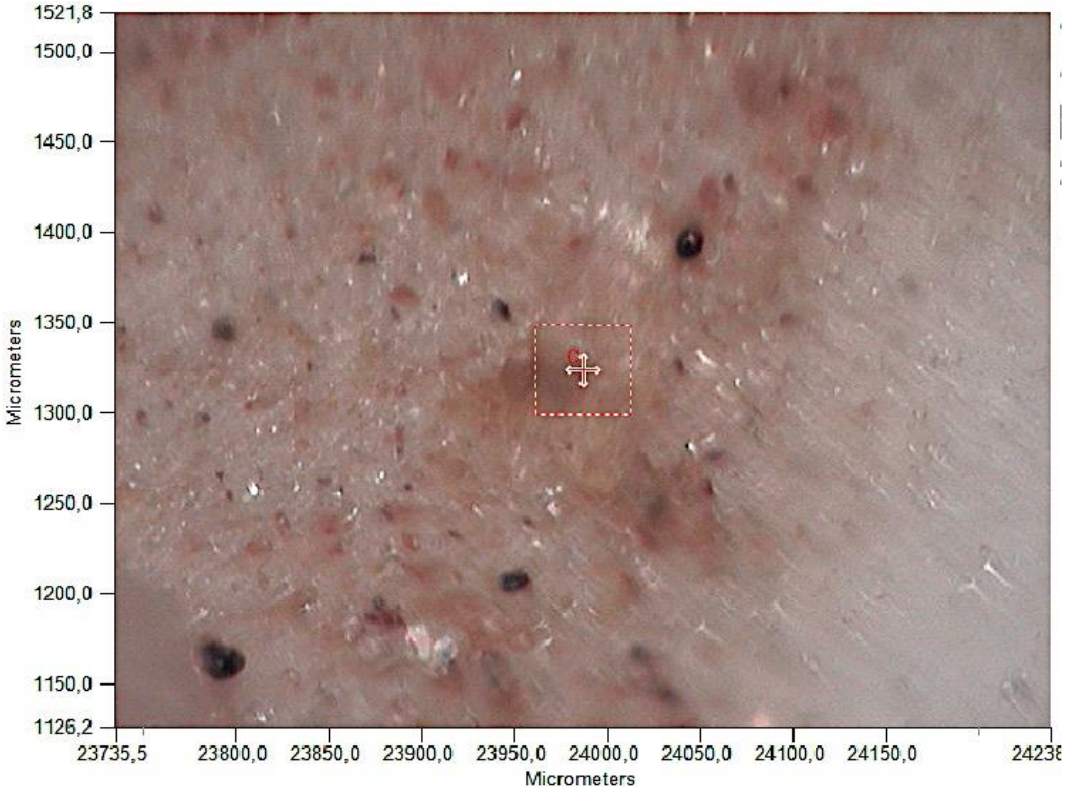

Figure 1. Illustration of PP MP square measurement in 50 × 50 µm found in mussel (*P. viridis*)

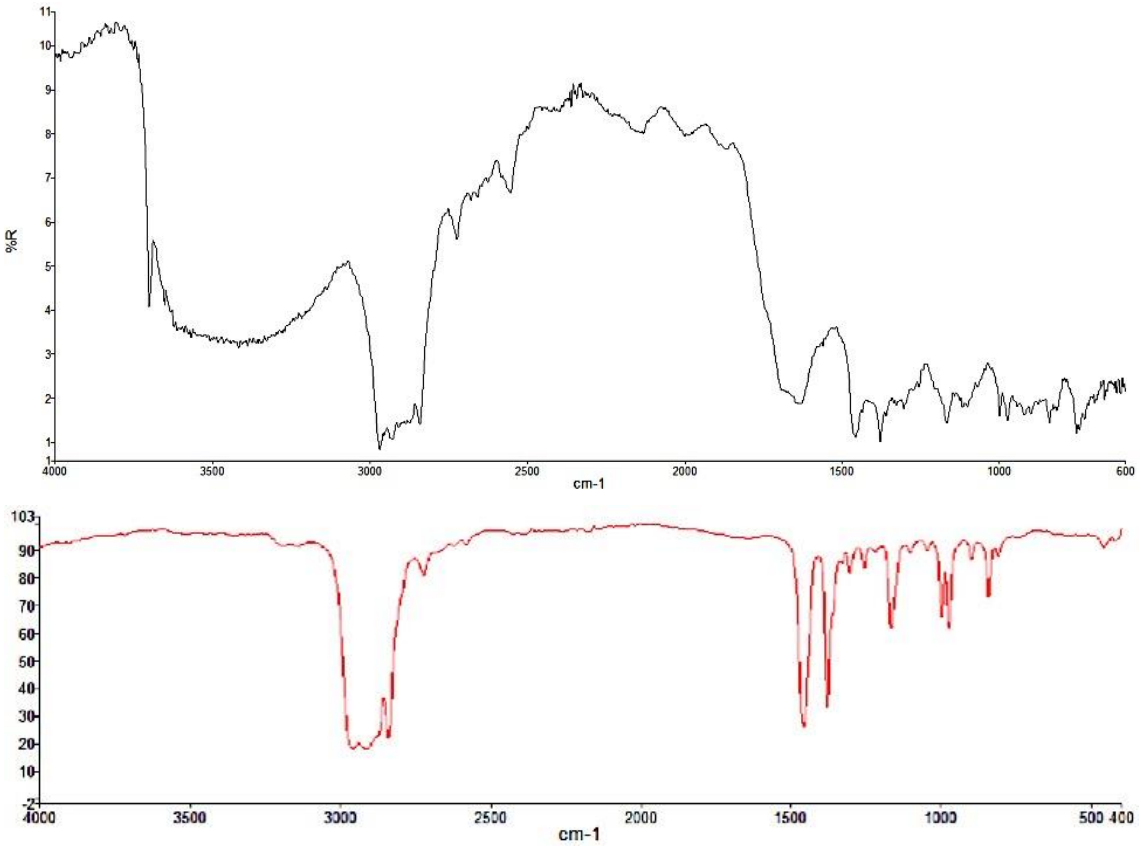

Figure 2. Spectrum of PP MP in black and PP reference in red

Table 1. Comparison of MP abundance in bivalves found in literature

Bivalves	Digestion	Identification	Quantity (MP/g ww)	Location	References
M. edulis	H_2O_2	Observation	34-178/indi	Canada	Mathalon and Hill, 2014
		µFTIR	0.9-4.6/g	China	Li et al., 2016
	HNO_3/ $HClO_4$	Observation	0.3-0.5/g	Belgium	De Witte et al., 2014
	HNO_3	Raman	0.36/g	Germany	Van Cauwenberghe and Janssen, 2014
			0.2-0.3/g	North Sea	Van Cauwenberghe et al., 2015
	Protease	Observation	2.5/g	England	Catarino et al., 2016
	KOH	µFTIR	0.24/g	France	Phuong et al., 2017
M. galloprovincialis	HNO_3	Observation	0.1-0.2/g	Europe	Vandermeersch et al., 2015
C. gigas	HNO_3	Raman	0.47/g	France	Van Cauwenberghe and Janssen, 2014
	KOH	µFTIR	0.18/g	France	Phuong et al., 2018b
P. viridis	H_2O_2	SEM/EDX	4-20/g	Indonesia	Khoironi and Anggoro, 2018
	H_2O_2	µFTIR	1.5-5.4/g	China	Qu et al., 2018
	KOH	µFTIR	0.29/g	Vietnam	This study

Regarding on the same protocol, the results in this study are more than mussel (*M. edulis*) and oyster (*C. gigas*) from of France (Phuong et al., 2018a; 2018b) for both of MP by the individual or by weighing. However, it's difficult for the comparison due to the difference of species analyzed, and particularly, from the difference of environment media. In fact, bivalves analyzed by Phuong et al., (2018a, b) were sampled in

seawater zone in France while in this study from a brackish zone in Vietnam. Although the KOH 10% solution is still ideal as digest reagent for bivalve's tissues but the difference of organic/inorganic matters in bivalves leads to different on filtration step. While the bivalves sampled in seawater filterable on a filter of 25 mm in diameter, the filter utilized in this study is 47 mm in diameter (same porosity of filter and tissue weigh at the beginning). According to our knowledge, the presence of alluvium in habitat (from the aquaculture ponds) at high quantity leading cause the difficulty for filtration step.

3.2. MP Characterization

Polypropylene (PP) and polyester have found more between six MP types in bivalve (Fig. 3). Some previous studies demonstrated that the PP and PE were predominant in bivalve (Phuong et al., 2018b). These two polymers types are usually utilized in household materials with short lifetime (*i.e.* packing bag for the PP and clothing for PE).

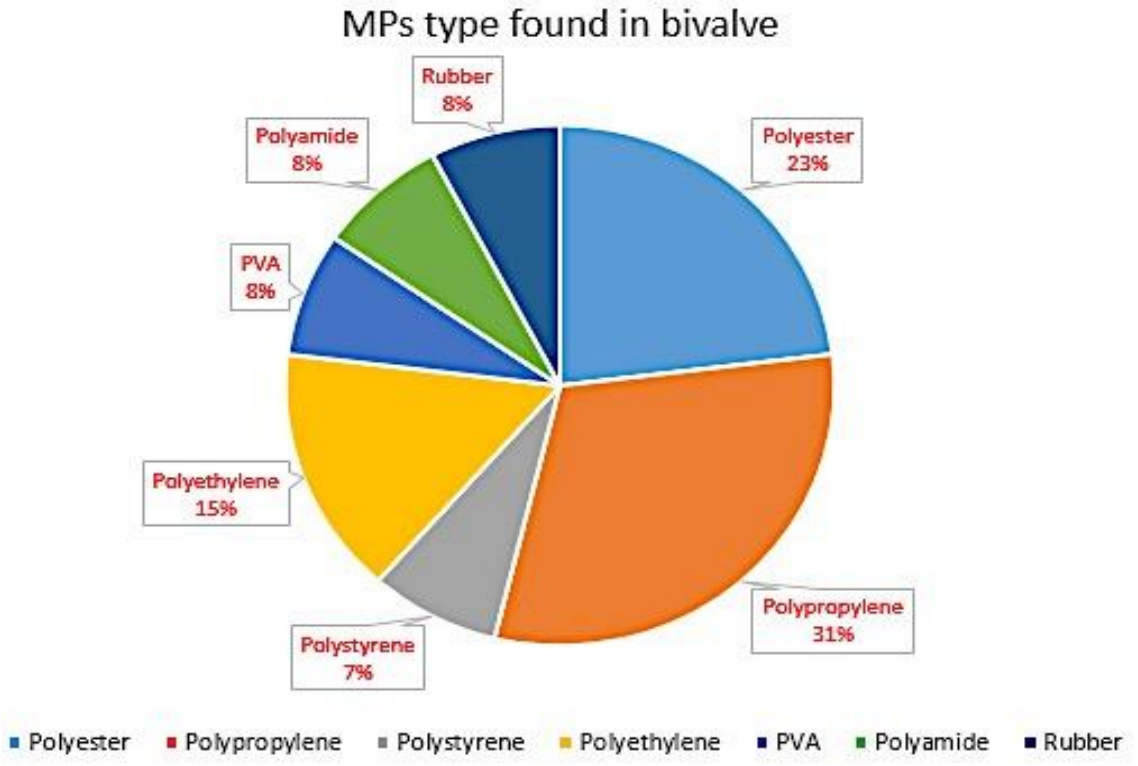

Figure 3. Types of MP were found in bivalve in Thanh Hoa province in Vietnam

A hypothesis that these polyester MP from clothing seem to accord with the MP form observed. It means that two out of three (66.7%) polyester MP were in fiber form. Around 70% MP was found in fragment form which seems high agree with other studies because that MP secondary represented as majority source of MP in the environment. Besides, we found also any MP in sphere form which permits to suggest that MP primary present also in MP contamination in bivalves from of Vietnam.

MP sizes observed in this study between 15 to 400 µm seem to highly agree with the results observed in Phuong et al., (2018a, b). However, they were larger than MP size

found in the study of Van Cauwenberghe and Janssen (2014). It could be explained by the different technics used. While the Raman spectroscopy found the MP between 5 to 25 µm, the µFTIR found the MP between 20 to 400 µm. It could be attention that the use of Raman spectroscopy usually needs much time for analysis. On another aspect, four types of colors have been observed with a high proportion of grey to light-grey. This observation demonstrated that these MP have passed longtime in the environment.

4. Conclusions

The contamination of MP in mussel (*P. viridis*) from Vietnam was preliminarily investigated in this study. The MP concentrations and characterization in mussel *P. viridis* in Vietnam were reported about 0.29 (±0.14) MP/g of wet weight and 2.60 (±1.14) MP/indi (N = 5). Firstly, it permits to contribute to the global MP contamination. We also compared the MP contamination in bivalve from different countries. However, this study is still limited because it is based on only one species at one sampling site in Vietnam, thus, more studies need to be invested in future (i.e. protocol's optimization for each species, each environment media). In more details, it needs also the studies for different environmental compartments and different species (water, sediment, organisms) in different zones in Vietnam for assessing the right MP pollution level in Vietnam, contributing to the global view of MP contamination. So far, another study on the toxicology of MP (with or without of contaminants such as HAP, PCB, DDE) is also required in parallel for allowing to evaluate the vector role of MP, especially their impacts on human health.

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