

## EFFICIENCY ASSESSMENT OF MICROPLASTIC EXTRACTION FROM GREEN MUSSEL *Perna viridis* Linnaeus

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### ABSTRACT

Microplastics with particle size less than 5 mm are becoming a raising global environmental crisis. These pollutants were found from the poles to the equator, in continental shelves, coasts and in the oceans, moreover, they have also been identified in the water columns, sediments and even in a variety of organisms. The majority of microplastics that ended up in the oceans originate from the land. Due to their small size, they are easily accumulated in the food chain, causing harmful effects on organisms and human health. The bivalves especially caught the interest of scientific researchers because of their direct contact with microplastics through the filter-feeding habit. Therefore, it is essential to develop methods to determine the presence of microplastics in these organisms and identify their source. This study evaluated the efficiency of extracting microplastics from the tissues of green mussels (*Perna viridis*) using KOH 10% solution to digest and KI 50% as the separating solution. Mussel soft tissue samples were spiked five different types of microplastics: polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polypropylene (PP), high-density polyethylene (HDPE) and treated with KOH 10% solution and KI 50% solution. The presence of microplastics in some green mussel species was also investigated in some mussel farming areas in Giao Thuy, Nam Dinh province, Thi Nai, Quy Nhon, Binh Dinh province and Hue city, Thua Thien Hue province. The research results showed high efficiency of microplastic extraction and recovery with the range from 76% to 97%. Microplastic concentration obtained in all mussel samples varies from  $1.0 \pm 0.1$  particles/g to  $1.7 \pm 0.6$  particles/g, in which fiber microplastics predominated. Microplastics in mussel samples have small sizes of  $< 1,000 \mu\text{m}$  and  $1,000\text{--}2,000 \mu\text{m}$ , make up 74.15–82.32% and 9.76–14.71%, respectively. Purple was dominant among all mussel samples. This study proved that using KOH 10% solution and KI 50% solution to isolate microplastics is a suitable approach and can be used in monitoring studies of microplastic pollution in bivalves.

**Keywords:** Bivalves, digestion, microplastics, green mussel, *Perna viridis*.

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## INTRODUCTION

In recent years, the occurrence and accumulation of microplastics in the air, aquatic environment or in sediments of ponds, lakes, rivers and seas,... is one of the environmental issues that receive a lot of attention from the researchers. Because microplastics are usually very small (less than 5 mm), accumulation in organisms is often observed. This is also a toxicological concern because plastics are known to contain a wide variety of additives or adsorb high concentrations of organic pollutants (Teuten et al., 2009). Therefore, it is crucial to establish standardized methods to detect microplastic accumulation in organisms to assess exposure, facilitate cross-study comparisons and enable robust risk assessment of microplastics in the environment. This problem has been emphasized by the International Council for the Exploration of the Sea (ICES) on plastic monitoring in organisms (ICES, 2015).

Sveral studies suggested that bivalve molluscs are bioindicators for microplastic exposure assessment, in the same way, they are used as bioindicators for other environmental pollutants (Li et al., 2016; Nam et al., 2017). The research group of Von Moos et al. (2012) demonstrated that *Mytilus edulis* L. can feed on high-density industrial polyethylene (HDPE) microparticles, > 0-80 µm in size, into cells, tissues, stomach and gastrointestinal tract after 3 hours of exposure. Digka et al. (2018) reported that in mussels *Mytilus galloprovosystemis* there was a presence of microplastics with a frequency of 46.25% and ranging from 1.7–2 items/individual. Most of the microplastics ingested by mussels are fragments, while their color and size vary widely. The study result of the green mussel *M. galloprovosystemis* reported that the microplastic content in these mussel samples was about 1.06–1.33 items/g (wet weight) (Alessio et al., 2019). The size classification revealed a marked prevalence of particles smaller than < 1,000 µm and the most abundant polymers present in the organisms analyzed were PE, followed by PP,

PET, PS, PLY and PVC (Alessio et al., 2019). Overall, microplastics were found in both *Mytilus* spp. in nature and artificial farming, but different methods of quantifying and digesting soft tissue make comparisons difficult.

Li et al. (2016) used H<sub>2</sub>O<sub>2</sub> (30%) as a soft tissue organic matter digester and a saturated salt solution of NaCl to investigate microplastic contamination in mussels (*Mytilus edulis*) from 22 sites along 12,400 miles coast of China in 2015. The study results showed that the concentration of microplastics varied from 0.9 to 4.6 particles/g and from 1.5 to 7.6 particles/individual. Fibers are the most prevalent kind of microplastics, followed by fragments. The fraction of microplastics with a size smaller than 250 µm accounts for approximately 17–79% of total microplastics. Vandermeersch et al. (2015) also compared two organic decomposition processes in *Mytilus galloprovosystemis* using nitric acid (HNO<sub>3</sub>) and a mixture of 65% HNO<sub>3</sub> and 68% perchloric acid (HClO<sub>4</sub>) with the ratio HNO<sub>3</sub>:HClO<sub>4</sub> (4:1) and the research results show that the microplastic recovery efficiency is higher applying the acid mixture method. Other methods are also used to determine microplastic content in mussel samples in particular and bivalve samples in general, such as using a single or a mixture of strong acids (HNO<sub>3</sub>, HCl, HClO<sub>4</sub>) and base (KOH, NaOH) (De Witte et al., 2014; Van Cauwenberghe & Janssen, 2014; Claessens et al., 2013; Rochman et al., 2015; Dehaut et al., 2016). Nam et al. (2017) experimented using KOH and NaOH, HNO<sub>3</sub>, a mixture of HNO<sub>3</sub> with H<sub>2</sub>O<sub>2</sub>, HCl or HClO<sub>4</sub> as an organic decomposer in mussel *Mytilus edulis* and a saturated KI solution to separate microplastics. Research results revealed that the method of using 10% KOH had an organic removal efficiency of up to 99.9% and saturated KI solution had a PE, PVC and PP recovery efficiency up to 80%. With the method of using concentrated HNO<sub>3</sub> during tissue digestion, the microplastic concentration reported herein might be considered as low, since the techniques used to extract the microplastics might change the

shape of the microplastics or destroy the particles in the sample. Claessens et al. (2013) as well claimed that concentrated HNO<sub>3</sub> could destroy these microplastics during the extraction. The use of strong acids such as HCl or HClO<sub>4</sub> can also lead to the same conclusion: these methods could damage or destroy pH-sensitive polymers (polyamide, nylon). Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) has also been used to digest organic matter before extracting MPs, but the downside of this technique is the organics are not completely decomposed and the decomposition time is long (Mathalon & Hill, 2014).

The objective of this study was to accredit the efficiency of microplastic extraction with 5 common microplastics from the tissues of mussel *Perna viridis* by using KOH 10% solution and KI 50% salt solution. In addition, the level of microplastic pollution in green mussels collected in some localities was also surveyed and evaluated through the application of an assessed analytical procedure.

## MATERIALS AND METHODS

### Microplastics preparation

Five types of microplastics are prepared from common plastic products in daily life, including food containers (polystyrene, PS), wire covers (polyvinyl chloride, PVC), bottle caps (polyethylene terephthalate, PET), medicine boxes (polypropylene, PP) and bags (high-density polyethylene, HDPE) (Table 1). These plastic products are washed with alcohol and left to dry naturally under laboratory conditions. The plastics are then cut, crushed and sieved through a metal mesh to ensure the size is less than 500 µm, afterwards collected and stored in glass vials. The size of each piece of plastic was measured using a stereomicroscope (Leica MZ12 stereo microscope with 16-160X magnification) equipped with image analysis software (LAS software®). Microplastic fragments with sizes in the range of 300–500 µm were selected to be used in the experiment to evaluate the efficiency of microplastic extraction.

Table 1. Microplastics (polymer type, source, color) used in the study

| Polymer                          | Specific weight (g/cm <sup>3</sup> ) | Color  | Source          |
|----------------------------------|--------------------------------------|--------|-----------------|
| Polyvinyl chloride (PVC)         | 1.1–1.35                             | Black  | Wires           |
| Polyethylene terephthalate (PET) | 1.38–1.41                            | Orange | Bottle caps     |
| Polystyrene (PS)                 | 0.96–1.04                            | White  | Food containers |
| High-density polyethylene (HDPE) | 0.94–0.97                            | Green  | Bags            |
| Polypropylene (PP)               | 0.85–0.95                            | Blue   | Plastic boxes   |

### Mussel sampling and preparation

Samples of adult *P. viridis* green mussels used in this study were collected from natural aquaculture ponds in Giao Thuy, Nam Dinh province, Thi Nai, Quy Nhon, Binh Dinh province, and Lap An, Lang Co, Hue city, Thua Thien Hue province during May–June 2021. Immediately after sampling, each green mussel was wrapped with aluminium foil and stored at -20 °C until analysis.

*P. viridis* green mussel samples collected in Nam Dinh were used for experiments that evaluate the efficiency of microplastic separation from bivalves. Samples of green

mussel contaminated with microplastics were prepared as described above. The size of the shell, the weight of green mussels before and after shell removal were determined. Five green mussels after removal (soft tissue) were placed in separate 150 mL glass flasks (one individual/bottle) (n = 5). Ten microplastics of each type listed previously (5 types of microplastics, total 50 microplastics) were added to the glass flask containing the soft tissue of mussels, and KOH 10% solution was added to digest the organic matter then placed on a magnetic stirrer at 230 rpm and 60 °C for 24 hours. Microplastics are separated due to

the density difference. Then, the solution was put on the separating funnel to perform the density separation step using KI 50% solution. The upper part of the final solution containing microplastics was filtered by a glass filter using GF/A membrane (Whatman, 1.2  $\mu\text{m}$  size). Filters were kept in sterile Petri dishes until observed under a Leica S9i microscope. The shape and color of the microplastics were observed and measured using the LAS@ software (Nam et al., 2017). In addition, a batch of the green mussel samples collected from Nam Dinh without adding microplastic fragments and distilled water samples adding microplastics (5 types of microplastics, a total of 50 microplastics) were prepared and gone through the same procedure as above. The final determined number of microplastics allows assessing the separation efficiency of the analytical procedure.

Microplastic recovery efficiency = [microplastics collected and counted on the filter/infectious microplastics]  $\times$  100.

To evaluate the degree of microplastic pollution in bivalves, green mussels *P. viridis* collected in some areas such as Giao Thuy, Nam Dinh province (GT-ND), Thi Nai, Quy Nhon, Binh Dinh province (QN-BD) and Lap An, Lang Co, Hue city, Thua Thien Hue

province (Hue) (without microplastics) are treated according to the above process. Results were expressed as mean  $\pm$  standard deviation (SD) ( $n = 5$ ) and all statistical analyses were conducted using Excel 2016 and Original 8.0 software.

## RESULTS AND DISCUSSION

### Evaluation of microplastic recovery efficiency in mussels

The evaluation of the efficiency of microplastic recovery was carried out by adding 50 microplastics (PET, PP, PS, PVC and HDPE) to the distilled water sample (blank sample) and green mussel sample with KOH solution. The results of the study are presented in Figure 1. In the blank sample experiment, in general, the recovery efficiency of the 5 types of microplastics above reached 70%. In which, the highest was PET with the recovery efficiency rate of  $96.7 \pm 5.8\%$ , followed by PVC, HDPE and PP  $90 \pm 10\%$ ;  $86.7 \pm 5.8\%$  and  $83.3 \pm 15.3\%$ , respectively. The lowest was PS microplastic with a recovery efficiency rate of only  $76.7 \pm 15.3\%$ . In the research of Nam et al. (2017), in the control sample without including mussel soft tissue, similar results were obtained when using KOH 10% solution and KI 50% microplastic separation solution.

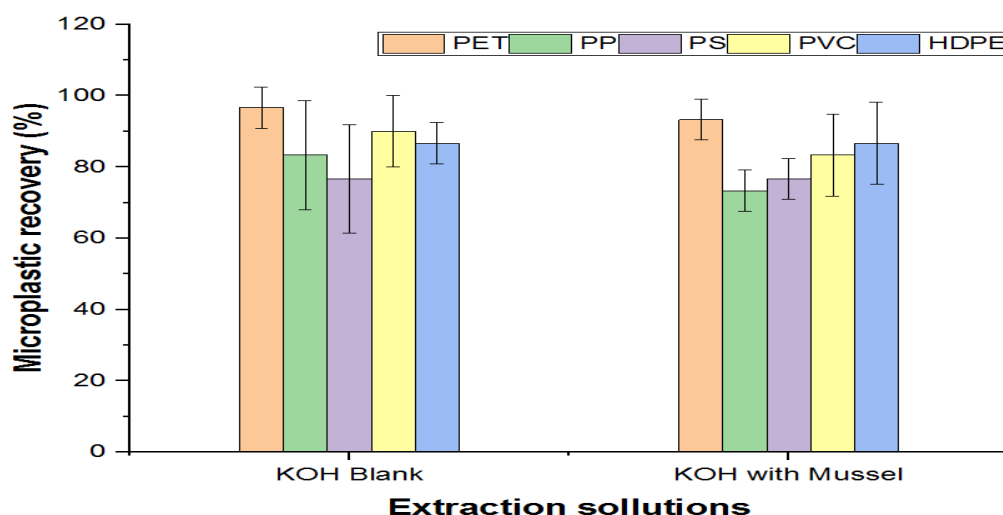


Figure 1. The recovery efficiency of 5 polymers (PET, PP, PS, PVC, PE, HDPE) in *Perna viridis* mussel soft tissue ( $n = 5$ )

Table 2. The microplastic recovery efficiency of in biological materials

| STT | Digesting solution                | Saturated salt solution | Temperature (°C) | The microplastic recovery efficiency (%) |          |                |                 |                  |                |                 | References          |
|-----|-----------------------------------|-------------------------|------------------|--|----------|----------------|-----------------|------------------|----------------|-----------------|---------------------|
|     |                                   |                         |                  | PA                                       | PE       | PET            | PP              | PS               | PVC            | HDPE            |                     |
| 1   | KOH 10%                           | NaI                     | 60               | -  | -        | 78.0<br>(2.49) | 102.0<br>(4.31) | 101.3<br>(8.51)  | 78.0<br>(2.49) | 103.8<br>(2.08) | Karami et al., 2016 |
| 2   | HNO <sub>3</sub> 69%              | NaI                     | 25               | -  | -        | 93.3<br>(5.46) | 86.3<br>(0.28)  | 87.9<br>(11)     | 68.9<br>(8.25) | 91.9<br>(4.62)  | Karami et al., 2016 |
| 3   | H <sub>2</sub> O <sub>2</sub> 30% | NaI                     | 50               | -  | -        | 99.5<br>(11.4) | 102.5<br>(9.20) | 105.4<br>(23.16) | 81.6<br>(5.77) | 99.5<br>(7.37)  | Karami et al., 2016 |
| 4   | HCl 37%                           | NaI                     | 50               | -  | -        | 89.6<br>(10.2) | 102.6<br>(8.76) | 100.7<br>(14.4)  | 78.5<br>(12.7) | 106.3<br>(9.37) | Karami et al., 2016 |
| 5   | KOH 10%                           | -                       | 60               | -  | -        | 87.5 (-)       | 81.5 (-)        | 90 (-)           | 85 (-)         | -               | Thiele et al., 2019 |
| 6   | H <sub>2</sub> O <sub>2</sub> 30% | -                       | 55-65            | -  | -        | 100<br>(-)     | 100 (-)         | 60 (-)           | 90 (-)         | -               | Digka et al., 2018  |
| 7   | KOH 10%                           | -                       | 60               | 100 (-)                                  | 100 (-)  | > 90 (-)       | 100 (-)         | 100 (-)          | > 90 (-)       | 100 (-)         | Dehaut et al., 2016 |
| 8   | KOH 10%                           | KI                      | 60               | -  | > 95 (-) | -              | 70 (-)          | -                | > 80 (-)       | -               | Nam et al., 2017    |
| 9   | KOH 10%                           | KI                      | 60               | -  | -        | 93.3<br>(5.8)  | 73.3<br>(5.8)   | 76.7<br>(5.8)    | 83.3<br>(11.6) | 86.7<br>(11.6)  | This study          |

Note: "-": None detected; The number in brackets represents the error results.

Table 2 describes the microplastic recovery efficiency in biological samples. In general, methods using solutions such as HNO<sub>3</sub> 69%, HCl 37%, H<sub>2</sub>O<sub>2</sub> 30% or KOH 10% are highly effective in soft tissue digestion of biological samples and recovery of microplastics (Karami et al., 2016; Thiele et al., 2019; Digka et al., 2018; Herrera et al., 2018; Nam et al., 2017). However, the study by Karami et al. (2016) reported on the limitations of some methods were declared: (1) long-term use of H<sub>2</sub>O<sub>2</sub> solution to decompose mussel tissue leads to partial dissolution and discolor of biological particles, changing the color of PET, degrade polymer structure, especially PVC and PS; (2) concentrated HNO<sub>3</sub> solution is the most destructive to various polymers such as melting LDPE, PP flakes and changing the color of most of the plastic polymers tested; (3) concentrated HCl solution causes fusion of PET particles resulting in reduced recovery efficiency. Corrosion of microplastic fibers, especially nylon fibers of strong acids such as HNO<sub>3</sub>, HClO<sub>4</sub> has also been investigated by Claessens et al. (2013). According to Karami et al. (2016) in terms of degradation efficiency, recovery rate and morphological changes of microplastics, strong acids and H<sub>2</sub>O<sub>2</sub> are not suitable candidates to separate microplastics from biological samples.

Dehaut et al. (2016) proposed a procedure using KOH 10% solution at 60 °C for 24 hours to extract and characterize microplastics from seafood tissues that overcome the above limitations. When applying this method to mussels *Mytilus edulis*, crab *Necora puber* and black seabream *Spondylisoma cantharus*, the soft tissue digestion efficiency reached from 99.6% to 99.8%. The KOH 10% solution was proven to have no effect on

polymers except cellulose acetate (Dehaut et al., 2016). Nam et al. (2017) also stated that this is a fast and reliable method for determining the level of microplastic contamination in bivalves. The output of this study also showed that the recovery efficiency of all kinds of polymers in *P. viridis* green mussel samples was up to 70%. In which, the highest PET recovery efficiency was  $93.3 \pm 5.8\%$ , followed by PVC, HDPE and PS at  $83.3 \pm 11.6\%$ ;  $86.7 \pm 11.6\%$  and  $76.7 \pm 5.8\%$ , respectively. The lowest was PP microplastic recovery efficiency with only  $73.3 \pm 5.8\%$ . Nam et al. (2017) also noted that using KOH 10% to degrade soft tissue of mussel *Mytilus edulis* also gave a high recovery rate of over 80% for PE, PP and PVC. Based on the above analysis, the method of using KOH 10% solution was evaluated as suitable and reasonable to decompose soft tissue and recover microplastic in the green mussel *P. viridis*. Therefore, in the next study, we investigated and evaluated the level of microplastic pollution in green mussels *P. viridis* collected in some localities.

### Microplastics in green mussel

#### *The concentration of microplastic particles*

In this study, the author's research team took samples of mussels at 3 different locations: Giao Thuy, Nam Dinh province, Quy Nhon, Binh Dinh province and Hue city, Thua Thien Hue province. At each location, 5 samples were taken to conduct the research. Shell length of mussels ranged around  $8.7 \pm 0.6$  cm. The soft tissue weight of mussels in this study ranged from  $12.4 \pm 4.3$  g at Giao Thuy, Nam Dinh province site to  $18.4 \pm 1.9$  g at the Hue city site, the average soft tissue weight of mussels was  $16.1 \pm 2.6$  g (Table 3).

Table 3. Average wet weight and shell length of mussels at each sampling site (n = 5)

| Location                          | Weight (g)           |                     | Length (cm)   |
|-----------------------------------|----------------------|---------------------|---------------|
|                                   | Before shell removal | After shell removal |               |
| Giao Thuy, Nam Dinh province      | $31.3 \pm 6.3$       | $12.4 \pm 4.3$      | $7.6 \pm 0.6$ |
| Quy Nhon, Binh Dinh province      | $33.5 \pm 6.7$       | $17.6 \pm 1.7$      | $8.9 \pm 0.2$ |
| Hue city, Thua Thien Hue province | $34.9 \pm 4.5$       | $18.4 \pm 1.9$      | $9.5 \pm 0.4$ |
| Average                           | $33.2 \pm 5.8$       | $16.1 \pm 2.6$      | $8.7 \pm 0.6$ |

The study results showing the microplastic concentration in the mussels are presented in Figure 2. Of the 15 analyzed green mussels, 100% of the green mussels contained microplastics. The concentration

of microplastics was found in mussels ranged from  $1.0 \pm 0.1$  items/g in Hue city,  $1.2 \pm 0.2$  items/g in Quy Nhon, Binh Dinh province and  $1.7 \pm 0.6$  items/g in Giao Thuy, Nam Dinh province.

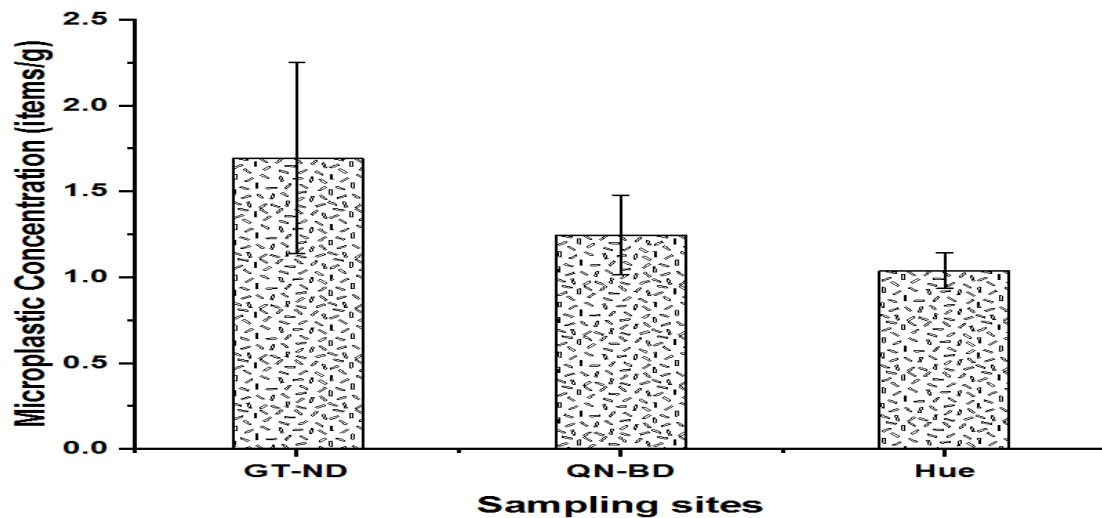


Figure 2. The concentration of microplastics in *Perna viridis* green mussels (n = 5) at 3 sampling locations

The initial results of this study also displayed the presence of microplastics in mussels in Vietnam. This provides warning signs for the coastal biota in the study areas. The concentration of microplastic particles in the mussels recorded in this study was lower than that of the green mussel (*Mytilus edulis*) samples in the marine environment in China (2.2 particles/g and 1.5–5.4 items/g) (Li et al., 2016; Qu et al., 2018) and higher than in some European countries (Vandermeersch et al., 2015).

#### Characteristics of microplastics

Observation of microplastic samples under a microscope with 100X magnification on Whatman GF/A filter paper showed that the shapes of microplastics in mussels at the three study sites were mainly fibers and fragments. Fibers accounted for the proportion from  $37.7 \pm 16.26\%$  to  $81 \pm 16.82\%$ , fragments proportion from  $19 \pm 16.82\%$  to  $62.33 \pm 16.25\%$  (Fig. 3).

In this study, there was a significant difference in microplastic shape at study sites. At Giao Thuy, Nam Dinh site, fragment microplastics mainly accounted in the green mussel samples with  $62.33 \pm 16.25\%$ . Meanwhile, fiber microplastics were found abundantly in the green mussel samples at Quy Nhon, Binh Dinh and Hue with  $81 \pm 16.82\%$  and  $79 \pm 12.47\%$ , respectively. Digka et al. (2018) demonstrated that fragments were the most abundant microplastic shape category in mussels with 77.8% and 22.2% were fibers.

Most studies have also reported that microplastics found in mussels are also mainly in the form of fibers and fragments, however, a higher percentage of fibers than fragments and fiber microplastics could account for up to 90% (Bråte et al., 2018). Research by Conrad (2020) also reported that fibers are the main microplastics found in 3 mussel species *Mytilus galloprovincialis*, *Choromytilus meridionalis* and *Aulacomya*

ater. Qu et al. (2018) also claimed that in *P. viridis* and *Mytilus edulis* fiber microplastics also predominate. This may be due to the shape of the microplastics when mussels absorb food through their filter gills (Wood et al., 2018). Li et al. (2019) also suggested that mussels absorb a lot of microplastics because bivalves are able to easily take them in through gills. Microplastics could trap in the gills, digestive glands and soft tissues which

leads to difficulty in removing these microplastics (Rocío et al., 2020). Lahens et al. (2018), Barrows et al. (2018) and Quinn et al. (2017) also demonstrated that fiber microplastics were quantitatively dominant in the urban rivers, ocean and sedimentary water samples. These causes lead to the high accumulation rate of microplastics in mussels as well as in invertebrates and vertebrates (Mizraji et al., 2017).

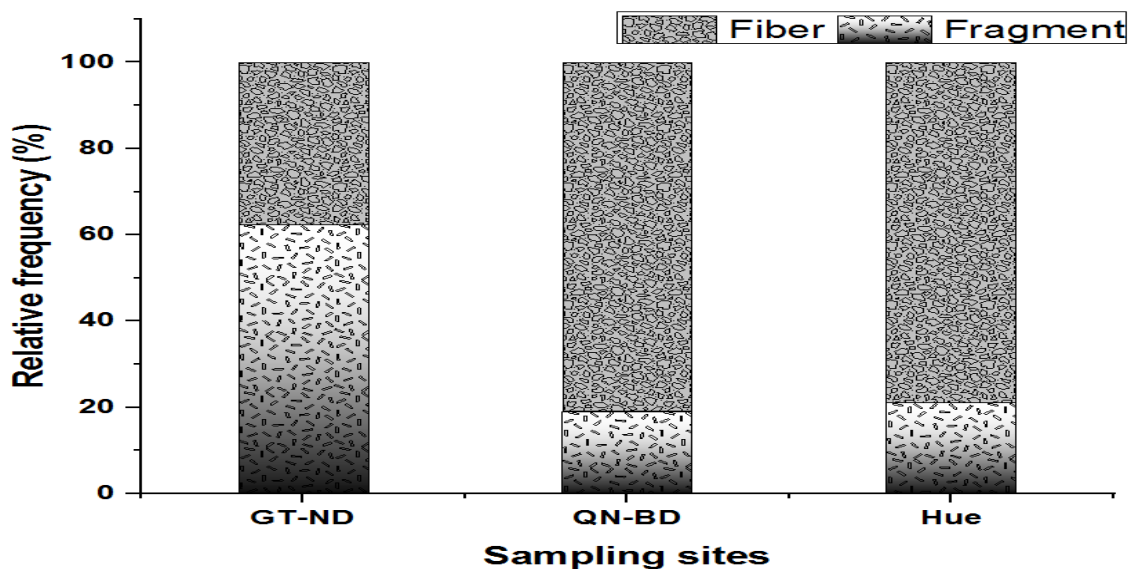


Figure 3. Percentage of microplastics in *Perna viridis* green mussel samples at 3 sampling locations

The difference in microplastic shape in mussel samples at Giao Thuy-Nam Dinh site compared with other sites may be related to different sources and waste management strategies in the area (Digka et al., 2018). In areas close to the sea where tourism and leisure activities are strong, if the solid waste management strategies will not suitable, it can lead to large amounts of plastic inputs from the sea or land (eg; plastic bags, plastic bottles, plastic cups) which break into microplastic fragments (Digka et al., 2018). In general, it is necessary to have further studies on the origin of microplastics as well as the relationship between the microplastics sources from water, sediment, soil and air to the

microplastic occurrence in mussels at these location studies.

The size of microplastic particles is closely related to the accumulation of microplastics in mussels. Therefore, this study also evaluated the size of microplastic particles in mussel samples at 3 study sites.

The size of fiber microplastics ranges from  $< 1,000 \mu\text{m}$  to  $6,000 \mu\text{m}$ . In which, mussel samples at 3 research sites Giao Thuy, Nam Dinh province, Quy Nhon, Binh Dinh province and Hue city, Thua Thien Hue province microplastics with the size  $\leq 1,000 \mu\text{m}$  accounted for the most with 82.32%; 74.15% and 76.67%, respectively (Fig. 4).



This might explain the easy encounter of microfibers in mussel species. Followed by microplastics with sizes in the range of 1,000–2,000  $\mu\text{m}$ , which also account for quite a lot with 9.76%, 14.71% and 13.33%, respectively. Microfibers with the large size 2,000–3,000  $\mu\text{m}$ ; 3,000–4,000  $\mu\text{m}$  and 4,000–6,000  $\mu\text{m}$  accounted for a very small percentage. Regarding the size distribution of mussel microplastics detected in this study, they are mainly divided into  $< 20,000 \mu\text{m}^2$ , 20,000–100,000  $\mu\text{m}^2$ , 100,000–200,000  $\mu\text{m}^2$  and 200,000–400,000  $\mu\text{m}^2$ . In which, the frequency of occurrence of microplastic fragments  $< 20,000 \mu\text{m}^2$  and 20,000–100,000  $\mu\text{m}^2$  in Giao Thuy, Nam Dinh province, Quy Nhon, Binh Dinh province and Hue city, Thua Thien Hue province locations

is 55.35%, 26.92%, and 50%; 38.57%, 31.62%, and 37.5%, respectively. Microplastics with sizes of 100,000–200,000  $\mu\text{m}^2$  and 200,000–400,000  $\mu\text{m}^2$  also appeared but with a very little rate. In general, the above data proved that in this study, microplastics in mussels were mainly fibers and that the fiber microplastic size was found to be dominated by microplastics smaller than 1,000  $\mu\text{m}$ . Research by Rocío et al. (2020) also reported that in the mussel *Limnoperna fortunei* 90% of the microplastics were also fibers and the most common size is  $\leq 1,000 \mu\text{m}$ . The studies by Bråte et al. (2018) also illustrated similar outcomes in the mussel sample *Mytilus* spp. mainly microplastics with a size less than 1,000  $\mu\text{m}$ .

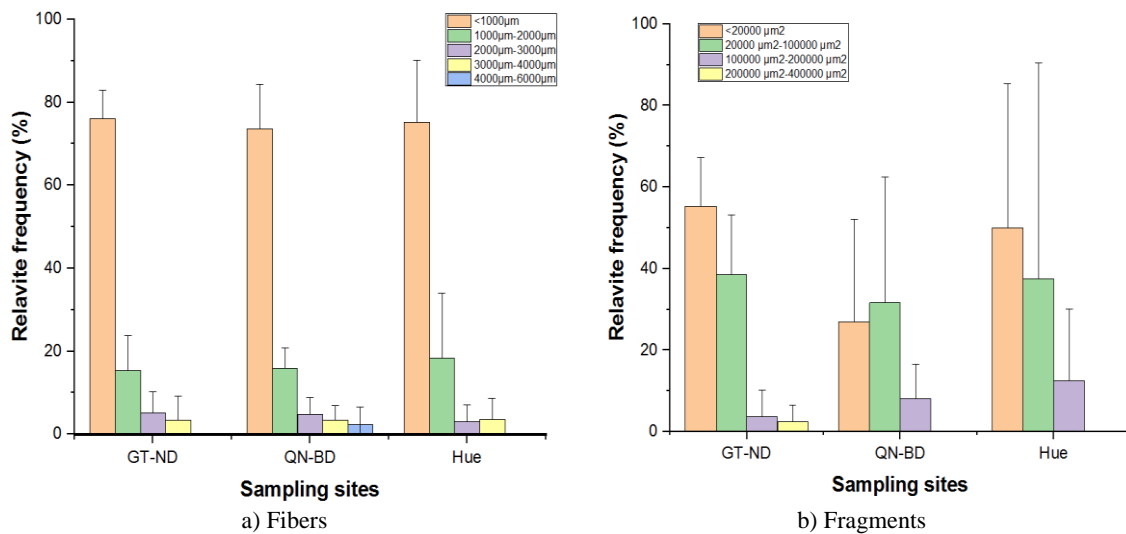


Figure 4. Size distribution of microplastic particles in mussel *Perna viridis* collected from 3 sampling locations

In this research, mussel microplastic samples were collected and sorted by color, with red, blue, white, black, green, yellow and purple colors (Fig. 5). Experimental data revealed that, in the mussel samples at 3 research sites, purple microplastic samples accounted for the highest percentage and had a large variation, specifically in Giao Thuy, Nam Dinh province 27.57%;

Quy Nhon, Binh Dinh province 34.32% and in Hue city, Thua Thien Hue province 47.36%. Green and blue microplastics also accounted for a high rate of 25.96%; 11.94%; 13.16% and 12.07%; 25.37%; 10.52%, respectively. In this study, there were as well microplastics colors of white 7.89–13.79%, red 5.17–11.94% and black 2.98–10.34%.

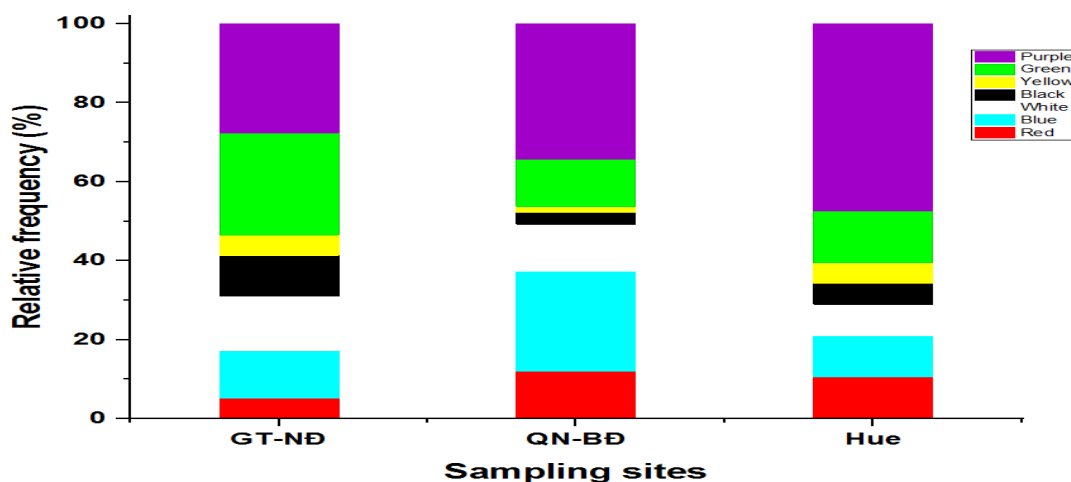


Figure 5. The color distribution of microplastics (MP) in *Perna viridis* mussels collected from 3 sampling locations

The results of this experiment confirmed that the color of the microplastic particles in the collected mussels is quite diverse. Colored microplastic particles in mussels account for a fairly large amount. In particular, microplastics with purple, green and blue colors were dominant, possibly because the mussel's habitat is affected by waste sources containing microplastics from clothing fabrics, plastic products, fishing gear,... (Su et al., 2016). In addition, colors such as white, blue, red, green and sometimes yellow have been observed. However, some studies have denoted that blue and dark blue fibers are more common in animal samples (Renzi et al., 2018; Duncan et al., 2019).

## CONCLUSION

Plastic pollution is a typical problem for marine life. This study has (1) evaluated the efficiency of microplastic recovery by using KOH solution to decompose soft tissues in mussels and KI saturated solution to separate microplastics and (2) evaluated the level of microplastics in mussels. This research presented a high efficiency in microplastics recovery from 76% to 97% using the above method and based on that helped to objectively assess the presence of microplastics in mussels. The concentration of microplastics found in mussels ranged from  $1.0 \pm 0.1$  particles/g in Hue;  $1.2 \pm 0.2$

particles/g in Quy Nhon, Binh Dinh, and  $1.7 \pm 0.6$  particles/g in Giao Thuy, Nam Dinh. There was a significant difference in microplastic shape at study sites. At Giao Thuy, Nam Dinh site, fragment microplastics mainly accounted in the mussel samples with  $62.33 \pm 16.25\%$ . Meanwhile, fiber microplastics were found abundantly in the mussel samples at Quy Nhon, Binh Dinh and Hue with  $81 \pm 16.82\%$  and  $79 \pm 12.47\%$ , respectively. Microplastics in mussel samples are mainly small particles of  $< 1,000 \mu\text{m}$  and  $1,000\text{--}2,000 \mu\text{m}$ . The results of this study might provide data for investigations and monitoring of microplastics in mussels and other aquatic animals.

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