



Preliminary Study and First Evidence of Presence of Microplastics in Green Mussel, *Perna viridis* from Phuket

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Abstract

Plastics can reach the coastal environment and could impact the marine environment. Mussels are marine organisms which are prone to be exposed to microplastics pollution. Therefore, in this preliminary study, the commercially important green mussel (*Perna viridis*) collected at the Koh Phee Canal in Phuket, Phuket Province, southern peninsular Thailand, was investigated. The collected mussels from aquaculture farm fell into three age groups, namely 1 year-1 month, 1 year-7 months, and 1 year-9 months. Results from the investigation showed 200 items of microplastics present in *Perna viridis* with 76 items (38%) in flushed water and 124 items (62%) in the soft tissue. The average counts of microplastics in soft tissue and flushed water were 4.13 items per individual and 2.53 items per individual, respectively. Blue color (98 items, 49%) and filament shape (185 items, 92.5%) were the most common color and shape of microplastics in the mussel tissues and the flushed water. The dominant polymers as microplastics were identified as polyester and polyethylene terephthalate, which are common polymer types used in protective packaging and containers. The statistical post hoc tests showed no significant differences by age group in the microplastics accumulation in the *Perna viridis* ($p < 0.05$). Further studies are required to understand the accumulation rates and residence times of microplastics across the food webs for better understanding on their impacts on human health. The results from this study provide a baseline level of microplastics contamination in green mussel aquaculture located at Phuket, and urgent measures are needed to prevent contamination of food for human consumption and related health problems.

Keywords: Environmental pollution; Environmental management; Environmental monitoring; Impacts on human health

Introduction

Plastic pollution is a growing concern in marine environment as it is the most abundant type of marine debris in the coastal areas [1–2]. A large portion of the plastic waste from urban and economic activities can reach the coastal environment [3]. Microplastics (diameter < 5 mm) introduced to the coastal environment can be transported over long distances and they are now considered a serious hazard to the marine environment [2]. The transfer of microplastics from land to sea and ultimately to the food chain is well-known to have a wide variety of impacts [4]. The potential for microplastics ingestion by a marine organism increases with decreasing particle size [5]. The transfer of microplastics may be by direct ingestion through accidental consumption by filter-feeding organisms [6]. Microplastics can act as a vector carrying chemicals, additives or organic contaminants, and could cause a wide range of sublethal and lethal effects in the ingesting organisms [7]. The impacts of plastic debris have been studied and reported [8–10]. Once ingested, microplastics can interrupt food ingestion and impact the marine organisms by reduced feeding capacity, detrimental alterations to intestinal function, tumor formation, immune response, and disrupted feeding [8, 10].

Many studies have identified microplastics in various marine organisms [11–13]. The accumulation of microplastics is demonstrated in various filter feeders such as *Balanus amphitrite*, *Littoraria* sp. [11], and *Mytilus* spp. [14]. Among the marine organisms, filter feeders such as clams, oysters and mussels can be considered bioindicators of marine pollution due to being widely distributed, having water-filtering capability, and their persistence to stay in the same place: these characteristics make them good recognized bioindicators in marine ecosystems [15]. The ability of green mussels to accumulate various types of pollutants makes them bioindicators used

to assess coastal pollution [16]. This characteristic is shared by several other non-bivalve species; however, the mussels are marine organisms which are prone to exposure to microplastics pollution because they have limited mobility and tend to stay in the same location; and have an ability to accumulate substantial amounts of pollutants from seawater into their tissues [16], and are susceptible to microplastics uptake while having a close connection with marine predators and with human health [17].

Phuket is in southern peninsular Thailand and has become important due to its rich natural resources, both for economic and ecologic considerations. Being a popular tourist destination, densely populated, and with rapid economic development, the waste generation causes environmental degradation. Previous studies have focused on heavy metal accumulation in the sediment and in marine bivalves (*Marcia optima*) species collected from the coastal area of Phuket Bay [18–19]. Most studies on microplastics contamination at Phuket have focused on the beach sediment, usually by tourist attractions [20–21]; however, there is limited literature available on microplastics contamination in marine organisms, especially in the marine bivalve *Perna viridis* that is an ecologically and commercially common marine bivalve species consumed by humans. In Phuket, the green mussel *Perna viridis* is considered one of the major aquaculture species, and it is the most consumed commercial species of bivalves in the fishery markets. Therefore, the aim of the present study was to assess microplastics accumulated in the green mussel *Perna viridis* sampled from a local aquaculture farm in mangrove creek, Koh Phee Canal, near Phuket town and a local market of Phuket, Thailand. Koh Phee Canal is in central Phuket at a very important location that connects Phuket city with coastal environment, especially the mangrove forest. This area is now becoming increasingly polluted by the contaminants discharged

into the canal from the city center of Phuket, and these emissions can be considered potentially significant sources of pollution to the local aquaculture farms, especially of plastic contamination from urban effluents. Green mussels (*Perna viridis*) can directly cause human exposure to microplastics as they are consumed whole, without gut removal. In addition to absorption, microplastics accumulation also occurs through attachment to the foot of a mussel [22]. The plastic size, shape, density, chemical composition and abundance greatly affect bioavailability in the environment, and together these characteristics determine the environmental impacts [23]. Therefore, microplastics in the sample of green mussels were analyzed both in the flushed water and in soft tissue. The present study will add to our understanding of the microplastics accumulation in commercial seafood and also support guidelines for food safety as the ingestion of microplastics by the green mussel *Perna viridis* can potentially cause serious health problems to humans [15–16, 24].

Materials and methods

1) Sample collection and preparation

The green mussel *Perna viridis* samples were caught or harvested according to the methods in [25]. The mussels collected were of different ages namely 1 year and 1 month (Group A), 1 year and

7 months (Group B), and 1 year and 9 months (Group C); and each age group was of one generation, continuing to grow, and kept on one and the same farm at the Koh Phee Canal, in Phuket Province. Group A (1 year and 1 month) is the average market-size that starts to be sold in the market; Group B (1 year and 7 months) was collected during the monsoon season and we assumed that the runoff from the land might cause microplastics contamination; while Group C (1 year and 9 months) is the oldest or the longest-lived mollusks before marketing them. A total of 30 samples, ten *P. viridis* mussel individuals from each age group, were randomly collected during March, September, and December 2019. The sampling location is illustrated in Figure 1.

The collected mussel samples were placed in aluminum foil bags and stored in an ice box for transport to the laboratory. The shell length and depth and width (cm) of each mussel were measured using a digital caliper (Senator, SEN-331–1330K). The shells were opened, and the whole soft tissue from each mussel was dissected and removed with forceps. The mussels were thoroughly rinsed with distilled water to remove the plastic particles from shell and tissues into a 600 mL beaker. Then, the tissue was rinsed and cleaned again by using distilled water into a 1,000 mL beaker before placing the tissue sample in the foil.



Figure 1 Study area.

2) Green mussel analysis

The analysis of microplastics in the green mussel *Perna viridis* followed the method of Li et al. [26] with some modifications. Approximately 20 ml of 1% KOH solution and 180 mL of 30% H₂O₂ were carefully added into the beaker for organic matter digestion. The beakers were covered with aluminum foil and left overnight, followed by 5 min in an oscillation incubator at 60 °C with a magnetic stirrer until no natural organic material was visible. To avoid contamination, all of the containers and beakers were previously rinsed three times with filtered water. Six grams of salt (NaCl) per 20 mL of sample was added to dissolve liquid of the soft tissue and to separate the microplastics from dissolved liquid of the soft tissue via floatation. The liquid was mixed, and the bottles were left to stand overnight. The overlying water was directly filtered with a 20- μ m-pore size filter, using a vacuum pump. Next, the filter was dried and placed into clean petri dishes with a cover for further analysis under a stereo microscope. Blank control group without any tissue was used for correction of potential procedural contamination.

3) Microplastics identification

Microplastics from each sample were counted under a Stereo microscope (Olympus-SZX7) with 40- and 100-fold magnifications and categorized into different 9 colors (white, transparent, red, black, blue, yellow, green, grey, and purple). The microplastics were further grouped into three shapes, namely, fibers, films, and fragments. The microplastics obtained from the flushed water and mussel tissues were analyzed using a Micro Fourier Transform Infrared Spectrometer (μ FT-IR) (Jasco FT/IR-6600) for the charac-

terization of the polymer type of a microplastic particle. The spectrum was recorded in the range from 4000 to 600 cm⁻¹ and analyzed with OPUS 7.5 spectral software. The composition of plastics was characterized by comparing the sample with the spectra library KnowltAll. In statistical analysis, the differences in quantities of microplastics between mussels and water were assessed by using SPSS (version 24). To determine the influence of mussel age on the accumulation of microplastics in the *P. viridis*, one-way ANOVA with a post hoc test was used.

Results and discussion

1) Characteristics of the green mussel *Perna viridis*

Averages of shell length (mm), shell width (mm) and depth (mm) of the mussels for each age group are given in Table 1. Very small differences in shell length, width and depth by age group were noted. According to the results across all age groups of the *Perna viridis*, the shell length ranged from 93.86 mm to 100.32 mm; the width ranged from 36.50 to 39.54 mm; and the depth ranged from 27.62 to 29.45 mm. Statistical analysis by the post hoc test showed that there were significant differences in the average shell length, width and depth of mussel samples ($p < 0.05$) between Groups A and C for length and depth, likewise between groups A and B for the width (Table 1). In mussel length, *P. viridis* at the age of 1 year and 1 month was significantly different from mussels aged 1 year and 9 months. In addition, *P. viridis* at the ages of 1 year and 1 month and 1 year and 7 months were statistically different for the shell width, and *P. viridis* at the age of 1 year and 1 month and 1 year and 9 months were different in shell depth.

Table 1 Physical characteristics of three age groups of *Perna viridis*

<i>Perna viridis</i>	Length (mm) (Mean±SD)	Width (mm) (Mean±SD)	Depth (mm) (Mean ±SD)
Group A: (1 year and 1 month)	93.86 ± 6.42 ^a	36.50 ± 1.82 ^a	27.62 ± 2.24 ^a
Group B: (1 year and 7 months)	96.81 ± 5.69 ^{ab}	39.54 ± 1.99 ^b	28.24 ± 0.98 ^{ab}
Group C: (1 year and 9 months)	100.32 ± 3.58 ^b	38.19 ± 2.22 ^{ab}	29.45 ± 1.85 ^a

Note: Significant difference at $p < 0.05$ level.

2) Amount of microplastics in green mussel, *Perna viridis*

The present study observed the presence of microplastics in both flushed water and soft tissue. The mean counts of the microplastics in the thirty samples of *Perna viridis* of the flushed water and soft tissue were determined and the numbers of microplastics (items) at different ages of the green mussel, *Perna viridis*, are given in Table 2. The total number of microplastic particles detected in *Perna viridis* was 200 items with an average of 6.67 items per individual, and that included 76 items in flushed water (38%); and 124 items found in the soft tissue (62%). The average counts of microplastics in the soft tissue and flushed water were 4.13 items per individual and 2.53 items per individual, respectively.

The results from the post hoc test showed that there were no significant differences by age group in the microplastics accumulation in the flushed water and in soft mussel tissue of the *Perna viridis* ($p < 0.05$). The highest average abundance of microplastics was found in the 1 year and 1 month group (Group A), while the lowest abundance of microplastics was detected in the 1 year and 7 months group (Group B) (Table 2). This revealed that the age of *Perna viridis* did not cause significant differences in the accumulation of microplastics, and this is probably because *Perna viridis* may be able to excrete foreign particles such as microplastics rapidly [27]. This study confirmed microplastics presence in *Perna viridis*, and urgent measures

are needed to prevent contamination of food for human consumption and related health problems. The observed accumulation of microplastics particles in green mussel, *Perna viridis*, might directly pose a risk to human health. However, the extent of accumulation of microplastics in humans through the food web depends on their rate of elimination and concentration in the edible tissues of biota [28].

Importantly, when compared with other studies, the amount of microplastics in *Perna viridis* in this study was similar, for example, to the average number of microplastics in the commercial green mussels in fishery market in Singhanakorn District, Songkhla Province, which varied from 5 to 30 items per individual [29]. Bajt, O. (2021) reported the average number of detected particles in mussel ranging from 2.6 to 12 items per individual [30]; and the number of total microplastics in mussels (*Mytilus edulis*) from the coastline of China varied from 1.5 to 7.6 items per individual [25], and from 0.4 to 5.0 items per individual in Asian clams (*Corbicula fluminea*) collected from Taihu Lake, China [31]. Blue mussel (*Mytilus edulis*) and cockle (*Cerastoderma edule*) on the Channel coastlines, France exhibited 0.76 to 2.46 items per individual [32]. In addition, microplastic and mesoplastic particles were also found in mussels collected in the South-West coast of England ranging from 1.43 to 7.64 items per individual [33]. These high levels of ingestion of plastic particles were linked to areas that were highly polluted with plastics [30].

Table 2 The average numbers of microplastics (item counts) in three age groups of *Perna viridis*

<i>Perna viridis</i>	Average count of microplastics (items)		
	Flushed water (Mean±SD)	Soft tissue (Mean±SD)	Total of microplastics (Mean±SD)
Group A: (1 year and 1 month)	2.6 ± 2.37	5.8 ± 2.15	8.4 ± 3.50
Group B: (1 year and 7 months)	2.8 ± 1.23	2.70 ± 1.77	5.5 ± 2.51
Group C: (1 year and 9 months)	2.2 ± 1.81	3.9 ± 3.00	6.1 ± 3.51

3) Microplastic characteristics in green mussel, *Perna viridis*

In this study, the colors of micro-plastic debris in the green mussel, *Perna viridis*, came in a great variety including white, transparent, red, black, blue, yellow, green, and grey, in both flushed water and in mussel tissue (Figure 2). The ranking by total counts of detected microplastics in both flushed water and tissue was blue > black > transparent = red > green > white = yellow > grey. Colors of microplastics from the flushed water showed that blue (41 items) dominated in this study, followed by black (11 items) and red (7 items), while in the mussel tissue, blue was the majority color (57 items), followed by black (18 items), transparent (13 items) and green (13 items). Blue-shaded microplastics were dominant in the detected microplastics of this study (49%, n=98 items), contributing almost half of the total cases. It was reported that blue is the most common color observed in the gastrointestinal tracts of other marine organisms [34] and in farmed mussel tissues [35]. In this study, blue was the prevalent microplastic (49%) observed in both flushed water and soft tissue of *Perna viridis*, and probably originated from the use of plastic fishing gear in mussel farming and mariculture activities around the canal. By comparison, studies of marine organisms from other regions in Thailand have indicated consistent results: for example, Chinfak et al. (2021) showed that blue and white were the two most frequently observed colors, in the two commercial bivalve

species, the *Perna viridis* and lyrate Asiatic hard clam (*Meretrix lyrata*), grown in the Tapi-Phumduang River and Bandon Bay, Surathani Province, Thailand [35]. Tokhun and Somparn (2020) also found contamination by blue microplastics in buffet food, and in mussel and fish tofu samples at three local markets in Ayutthaya and Pathum Thani, Thailand [36].

The distribution of microplastic debris by particle shape is shown in Figure 3. From the flushed water and soft tissue samples, it was found that filaments represented 92.5% (n=185 items), followed by fragments (7%, n=14 items) and sheets (0.5%, n=1 item). The shapes of microplastics can be ranked as follows; filament (87%) > fragment (9%) > sheet (1%) in the flushed water; and filament (96%) and fragment (4%) in the soft tissue. It is suggested that the synthetic fibers ingested by mussels might originate from plastic ropes used in fishing nets or in plastic lines where farmed mussels are grown [37]. From the reviews, the majority of the microplastics ingested by marine organisms are filaments, for example, Su et al. (2018) reported that microfibrils were the most dominant types of microplastics found, accounting for 60 to 100% in Asian clams (*Corbicula fluminea*) collected from Taihu Lake, China [30]; and Lusher et al. (2017) found that blue mussels had fibres (85%), fragments (11%) and films and foams (4%) [38]. Review of the distribution of microplastics in marine organisms among various regions in Thailand is also given in Table 3.

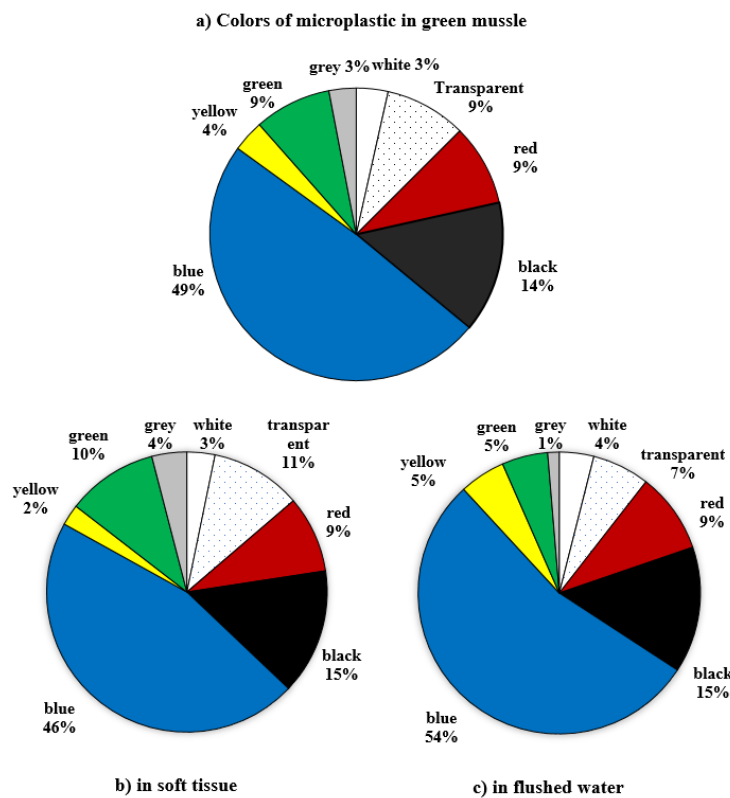


Figure 2 Colors of microplastics by particle count (%) in a) green mussel (*Perna viridis*); b) soft tissue; and c) flushed water.

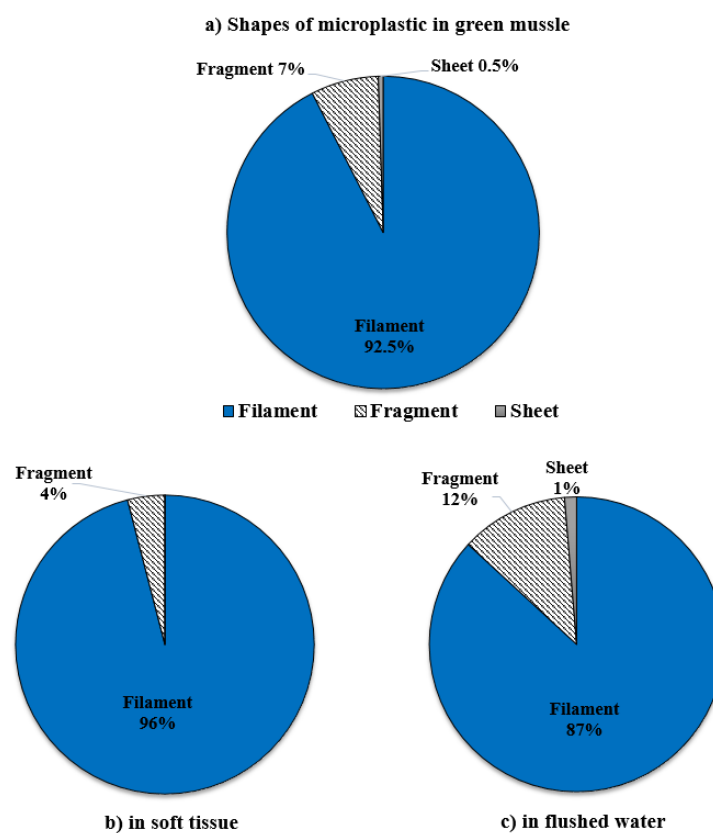


Figure 3 Shapes of microplastics by particle count (%) in a) green mussel (*Perna viridis*); b) soft tissue; and c) flushed water.

Table 3 Reviews of microplastics distributions in marine organisms from various regions in Thailand

Chinfak et al., 2021 [35]	Blue and white were the two most frequently observed colors in the green mussel (<i>Perna viridis</i>) and lyrate Asiatic hard clam (<i>Meretrix lyrata</i>), grown in the Tapi-Phumduang River and Bandon Bay, Surathani Province, Thailand. Rayon was the most prevalent polymer (40% to 55%), followed by polypropylene (PP) or polyethylene (PE), polyethylene terephthalate (PET), and nylon. Fibers were detected in mussels (100%), and clams (95%).
Tokhun and Somparn, 2020 [36]	Microplastics in buffet food at three local markets in Ayutthaya and Pathum Thani, Thailand were mostly fibers (57.7%), followed by fragments, microbeads, rods and pellets, in rank order. The highest contamination was observed in mussel and fish tofu samples (16 pieces/5g of tissue, wet weight).
Klangnurak et al., 2020 [13]	Microplastics (27.27%), mesoplastics (69.88%) and macroplastics, (2.85%) were found in fish collected from the lower Gulf of Thailand and the ingested plastics were mostly fibres.
Goh, et al., 2019 [29]	Microplastics counts in green mussel (<i>Perna viridis</i>) sold in fishery market in Singhanakorn District, Songkhla Province, were 12.30 ± 0.20 items per individual. The size of microplastics ranged from 0.18 to 4.2 mm with 0.5 to 1 mm being the most common size class. Fibres were the dominant shape (95.9%) of total microplastics, with high abundance of black and blue microplastics discovered.
Azad et al., 2018 [12]	Microplastic fibers and polyamide were the dominant shape and polymer type found in both demersal (82.76%, 55.17%) and pelagic fish (57.14%, 50.00%). The dominant microplastics color in both demersal and pelagic fish was red (31.03% and 28.57%, respectively).
Thushari et al., 2017 [11]	Microplastic in Rock Oyster (<i>Saccostrea forskalii</i>) and in Striped Barnacle (<i>Balanus Amphitrite</i>) collected from the beaches at Chonburi Province, on the eastern coast of Thailand, were found at 0.37 to 0.57 particles/g and 0.23 to 0.43 particles/g, respectively.

4) Identification of polymer type in green mussel, *Perna viridis*, by μ -FTIR

All the suspected microplastics (n=482 items) counted by microscopy (included 200 plastic items and 282 non-plastic items) were analyzed for their polymers by μ FTIR based on matching with the spectral database. The majority of the polymer types and non-plastic are given in Table 4. In the present study, 58.5% (n=282 items) of the particles identified by μ FT-IR spectroscopy were not plastic (35.5%, n=171 items and 23%, n=111 items of the particles in soft tissues and in flushed water, respectively). In total 200 items of particles were identified as microplastics, and are composed of a variety of polymers including polyester, polyethylene terephthalate (PET), urea-formaldehyde (UF), rayon, and etc. The main polymer types from the μ FTIR analysis in flushed water were identified,

as in the order polyester > polyethylene terephthalate > polyethylene with a great amount of cotton and polysaccharide also detected. In soft tissue of *Perna viridis*, polyester, PET, and urea-formaldehyde were dominant.

The ranking of the main polymer types in this study was polyester > PET > UF > Rayon > PE > PA > PS etc. The chemical composition of the microplastic particles in green mussel, *Perna viridis*, clearly shows the dominant presence of polyester and PET among the different types of polymers (Figure 4), and these are common synthetic plastics used in protective packaging, containers, lids, containers/bottles for beverages (juice, water, beer), detergents, plastic film, and microwavable packaging [30, 39–40]. UF is used as decorative laminates, textiles, cotton blends, and electrical appliances, while rayon is a synthetic fiber, made from

regenerated cellulose, and has been commonly reported in the marine environment. This type of fiber is common in fabrics popular for making clothes, curtains, tablecloths, home decorations, personal hygiene products, and industrial textiles such as mechanical rubber goods [41]. PE is the most widely used thermoplastic polymer for fabricated parts and components including chemical holding tanks, food processing parts, medical equipment, packaging applications, conveyor wear strips, piping systems, and liquid dispensing equipment [42]. Polyamide or PA is commonly known as nylon and is used in several application segments such as electronics, packaging, fishing lines, toothbrush bristles, and tubing [30]. PS is used for food containers, disposable cups, plates, cutlery, plastic tableware, take-out food containers, and compact disk cases [30].

Green mussel *Perna viridis* is commercially farmed using the hanging rope culture method and suspended from a raft at a varied depth of 0.5 to 1.0 m from the water surface [43]. Organisms that live in surface waters are likely to encounter microplastics with a density lesser than that of seawater, such as PS, PP, and PE, while benthic organisms that live at the lowest level of a water body (such as a lake or ocean) are more likely to encounter the denser PET or PVC that settle to the bottom [44]. This is the first report of a study of polymer types present in the green

mussel, *Perna viridis*, collected from Phuket Island in southern Thailand. Microplastics with a density lesser than that of seawater could float on the seawater column and have a potential to accumulate in the mussel. Polyester, PE and PET were the polymer types with the largest particle counts, and they are general-purpose thermoplastic polymers. The high counts of these polymers in the present study indicate a correlation between the contribution of microplastics and aquaculture activities and/or farming practice. It was reported that one of the main growing techniques is the use of aquacultural seed bags for a controlled growth, and they are made of polyester mesh [45]. The ropes and rafts are potential sources of microplastics for the farmed mussels. However, other fibrous microplastics used in the mussel farming sector should be further investigated to ascertain the sources of microplastics contamination in the aquaculture farm [46]. The results of this study are inconsistent with [30], which reported that the most common polymers in the mussels and in seawater were, in descending rank order, PE, PP, and PET, with smaller but equal amounts of PS, PA, and PVC. This is probably due to differences in anthropogenic pressures and culture conditions that might cause the differences in microplastics ingestion or in the deposition of various plastic polymer types.

Table 4 Polymer types of microplastics in flushed water and soft tissue of *Perna viridis*

Polymer types	Item (n)		
	Flushed water	Soft tissue	Total
Plastics	76	214	200
Polyester	26	44	70
Polyethylene terephthalate (PET)	20	38	58
Urea-formaldehyde (UF)	4	15	19
Rayon	8	5	13
Polyethylene (PE)	9	0	9
Polyamide (PA)	1	7	8
Polystyrene (PS)	2	3	5
Melamine	1	1	2
Polypropylene (PP)	0	1	1
Others (plastics)	5	10	15

Table 4 Polymer types of microplastics in flushed water and soft tissue of *Perna viridis* (continued)

Polymer types	Item (n)		
	Flushed water	Soft tissue	Total
Non-plastic	111	171	282
Cotton	83	115	198
Polysaccharide	8	19	27
Ink from kogia	1	11	12
Sodium lignosulfonate	5	4	9
Other (non-plastics)	14	22	36
Total	187	295	482

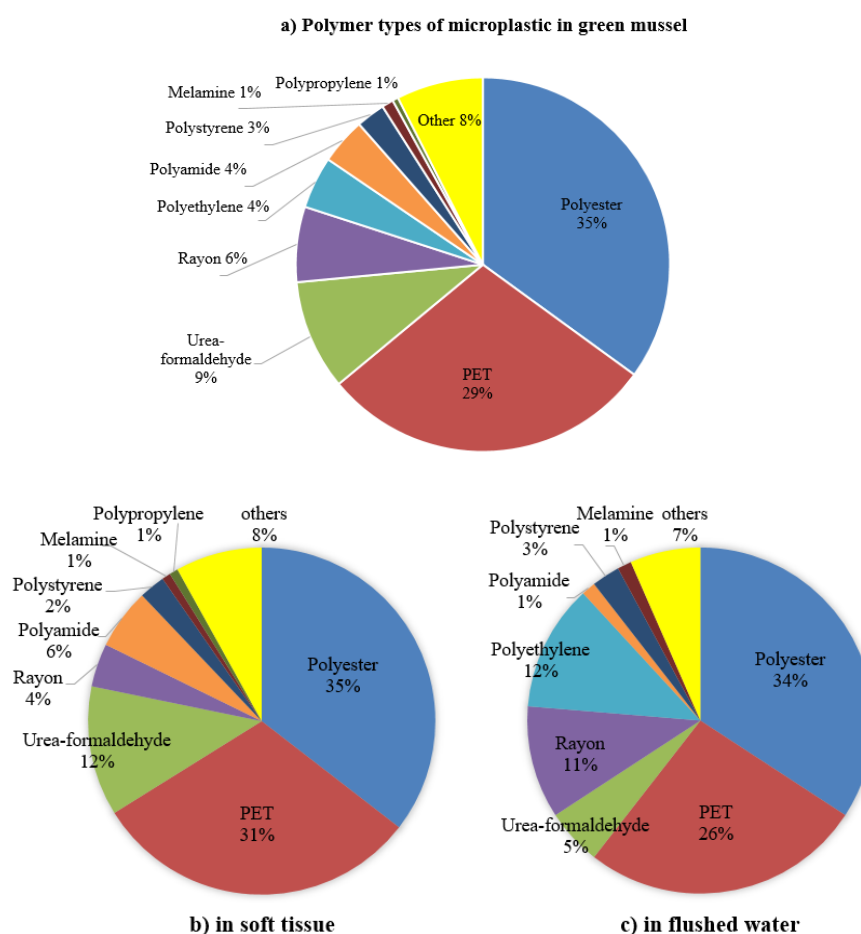


Figure 4 Types of microplastics by particle count (n = 200) in a) green mussel (*Perna viridis*); b) soft tissue; and c) flushed water.

Conclusions

Mussels have been widely used as bioindicators in the monitoring of coastal pollution, and this study has shown that microplastics are also present in the mussels (*P. viridis*). The occurrence of microplastics was observed both in the flushed water from the mussels and in their soft

tissue, and no difference in microplastics accumulation by age group of the mussels was found. Polyester and PET were the most common synthetic plastic polymers, and these are used in many packaging applications, and were dominant among the polymer types that had accumulated in the mussels. The ranges of polymer

type and color suggest a variety of potential sources. The microplastics were present in the flushed water of *P. viridis* in considerable numbers, so that the cleaning is an important step recommended in order to avoid transfer of the microplastics up the food chain, to accumulate in the human body from consumption of sea food. Further research is needed to understand the mechanisms influencing microplastics transport, deposition, resuspension, and subsequent interactions with biota. Large proportion of the mussels should be collected and studied as the relatively small mussel sample used in this study was a limitation that prevented a more comprehensive analysis. In addition, the authors suggest further studies on other types of bivalves and marine organisms in order to further assess the current condition of microplastics exposure, and thus, there should be a proper management system in order to reduce the effects of microplastics on organisms and their ecosystems.

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