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Microplastic Contamination in the Edible Tissues of Green Mussels Sold in the Fresh Markets for Human Consumption

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Abstract

The coastal and marine environment are currently polluted by microplastics (MPs) worldwide. The movement of MPs from land to sea and their incorporation into the food web has a significant negative impact on marine life and human health. The aim of this study was to quantify microplastics in soft tissue of green mussel *Perna viridis* (Linnaeus, 1758) sold in Nahkon Pathom and Salaya fresh market, Nakhon Pathom Province, Thailand. The total number of MPs was 30 items in mussels from Nahkon Pathom fresh market and 23 items from Salaya fresh market. The average content of microplastics was 0.51 ± 0.22 items/g (wet weight) in mussel sold in Nakhon Pathom fresh market, whereas in mussel sold in Salaya fresh market was 0.30±0.22 items/g (wet weight). Over half of the microplastics were 250-500 µm in size, and the most common shape was fibres (66%) and fragments (34%). The dominant color was blue (62%) and violet (38%). Polymer types were identified using FT-IR microscope, and the major component was polyethylene terephthalate (PET), polyvinyl alcohol (PVA) and polypropylene glycol methacrylate (PGM). Microplastic contamination was found in each soft tissue of green mussel individual. The findings indicated that the microplastic content of bivalve soft tissue was transferred to humans when they consumed whole soft tissue.

Introduction

Microplastics (MPs) contamination has become a major issue as a result of increased plastics production and poor waste management. Several researchers have investigated the presence of MPs in the air, soil, and aquatic environments in order to determine the scope of the problem. Plastic particles with a diameter of < 5 mm are considered MP, according to widely accepted definitions (Wright et al., 2013). The majority of studies on the presence of MPs in the environment have been conducted on marine ecosystems (Van Cauwenberghe & Janssen, 2014). Several researchers have found that MPs in the marine environment have an impact on a wide range of marine species (fishes, mussels, sea scallops, and seagulls) (Wegner et al., 2012; Gündoğdu et al., 2020). Because of the size of MPs, ingestion is the most typical way for MPs to enter marine species. Various studies are being conducted to better understand the presence of MPs in aquatic environments and the risks

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they pose. Mussels, oysters, and crabs were studied, as well as various methods for MPs to enter the human diet (Van Cauwenberghe & Janssen, 2014).

The composition of food in marine organisms is determined by their feeding strategies. This also determines the extent to which pollutants affect these organisms. For example, filter feeders (such as mussels and oysters) can consume everything in the surrounding water, implying that MPs can be consumed alongside microscopic life (such as copepods, decapods, and other planktons) (Walkinshaw et al., 2020). Many studies have shown that bivalves consume a significant number of MPs. However, no studies on the number of MPs in fresh market bivalves were discovered.

There is increasing concern about the impacts of MPs (< 1mm) on marine biota. The green mussel, *Perna viridis* (Linnaeus, 1758) (Mytilidae), is a commercially important tropical Indo-Pacific marine bivalve (Baker et al., 2007). Mussels are benthic extensive filter feeding organisms with a selective suspension feeding mechanism that accumulates microplastics, chemical pollutants, and microorganisms. Microplastics have been found in mussels from the wild as well as on farms in several European countries, as well as in the coastal environment of China (Mathalon & Hill, 2014; Van Cauwenberghe & Janssen, 2014; Li et al., 2015; Van Cauwenberghe et al., 2015; Li et al., 2016). According to recent reports, China's coastal and inland waters are a hotspot of plastic contamination in both biotic and abiotic factors (Qiu et al., 2015; Su et al., 2016). In the laboratory, various filter feeders were used to demonstrate the ingestion, accumulation, and translocation of synthetic microplastics (Farrell & Nelson, 2013; Van Cauwenberghe & Janssen, 2014) . Microplastics and nanoparticles are ingested or accumulated in the gut of mussels, resulting in the accumulation of plastic beads in the digestive system and haemolymph tissues. After being exposed to water, microbeads were discovered on the mussels' gills, indicating that not only sediment, but also plastic beads, could be trapped in the water column (van Moos et al., 2012). The presence of microplastics in mussel tissues and organs suggests that they are transferred to higher trophic levels, similar to what occurs in the human diet (Farrel & Nelson, 2013). In this present study, the green mussel *P. viridis* was purchased directly from two local fresh markets in Nakhon Pathom Province, Thailand. The purpose of this study was to conduct a preliminary investigation into the presence of MPs in the mussel population of a local fresh market.

Materials and methods

1. Sample collection and processing

In October 2020, a kilogram of green mussel (*Perna viridis*) was purchases directly from the two fresh markets (Nakhon Pathom fresh market and Salaya fresh market), Nakhon Pathom Province Thailand. Each market was assigned three replicates (R1, R2, and R3). Each replicate had 3 individuals (n=3) in it. In the laboratory, the wet weight of the soft tissue was determined using a precision electronic balance.

The digestion of mussels was performed as follows. In brief, each individual was placed in a labeled 100 mL glass beaker that had previously been cleaned, and then approximately 20 mL of 35% H_2O_2 was added to each glass beaker (Ehlers et al., 2019). The beaker was wrapped in parafilm and shaken at 150 rpm for seven days, until the soft tissue was completely digested. The digestion time could be adjusted based on the digestive effect, and digestion was stopped when the solution became clear.

Each solution sample was transferred to a glass separation funnel, and 99% potassium formate was added until the solution reached 1.6 g mL^{-1} saturation (Ehlers et al., 2019). For at least three hours, the samples were kept at room temperature. A saturated solution allows for the separation of less dense particles, resulting in a layer of MPs floating upwards while undissolved organic residues and inorganic matter settle at the bottom of the bottle.

The samples were filtered through a nylon membrane filter (pore size 0.45; diameter 47 mm, Whatman) using a pressure filtration unit. Each filter was placed in a clean Petri dish, covered with aluminum foil, and dried for two days in a drying cabinet (50°C).

Each filter was then visually inspected for microplastics using a stereomicroscope (Leica EZ4E) and all microplastics were identified based on their color and shape. Fiber, sphere, film (thin and small layer), fragment (part of a larger plastic item), and sphere were the shapes used to classify microplastic particles. The presence of microplastics was documented (fragments, films and fibers, and spheres).

Selected particles from the mussel specimens were manually analyzed using a Hyperion 2000 FT-IR microscope equipped with a mercury-cadmium telluride detector (Bruker) in a wavenumber range of 4,000-600 cm^{-1} with 32 co-added scans and a spectral resolution of 4 cm^{-1}. The software used was OPUS 7.5, and the

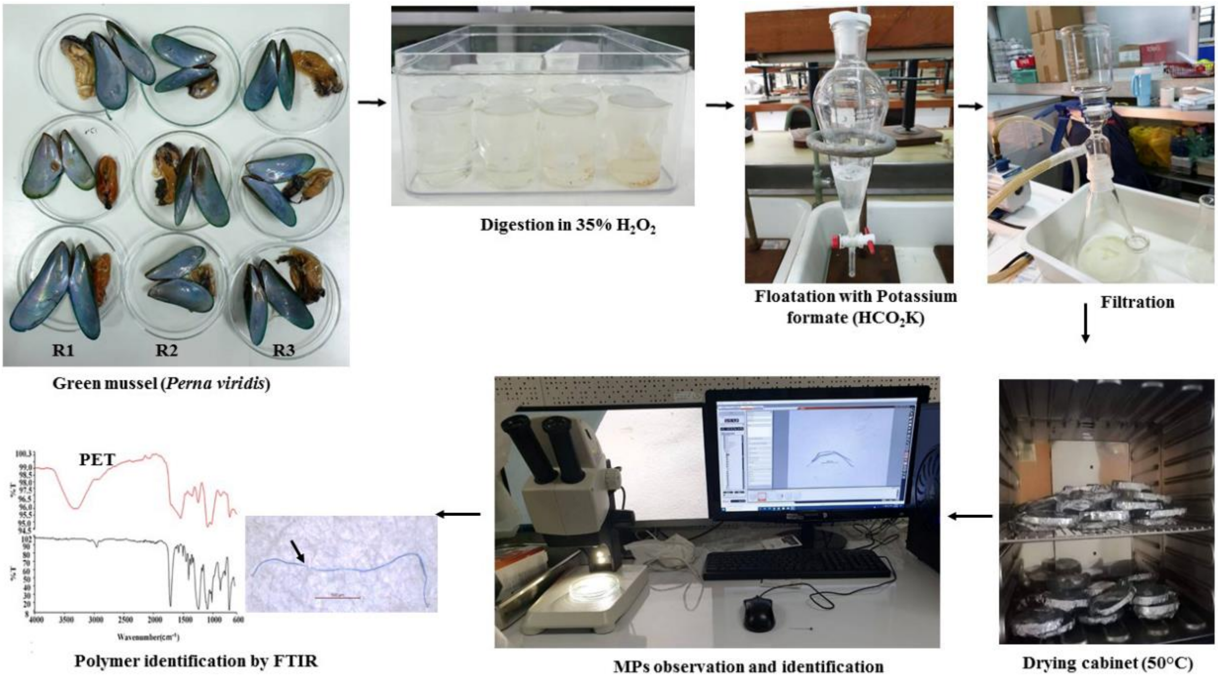


Fig. 1 Microplastics in green mussels: Sample preparation, digestion, and analytical procedures

resulting spectra were compared to the Bruker database. Only particles with a hit quality of more than 700 were considered microplastics, as in previous studies. The protocol is summarized in Fig. 1.

2. Data analysis

For each treatment, the type, size, and color of the MPs were measured and calculated. The average abundance of microplastic particles in mussel soft tissue was expressed in items/ g of tissue wet weight.

Table 1 Abundances of microplastics in the green mussels collected from two fresh markets. Three replicates were collected for each market (n=3)

Collection site	Microplastics (MPs) in soft tissues weight			
	Soft tissues weight (g)	Total MPs (items)	Average microplastic abundance (items/ individual)	Average microplastic abundance (items/g)
Nakhon Pathom fresh market	6.55±0.51	30	3.33±1.15	0.51±0.22
Salaya fresh market	8.41±1.10	23	2.56±1.90	0.30±0.22

Results and discussion

1. Abundance of microplastics in green mussels

The findings of this study confirmed that the green mussel sole in both fresh markets were heavily contaminated with microplastics (Table 1). All tested mollusk samples contained a total of 53 microplastics.

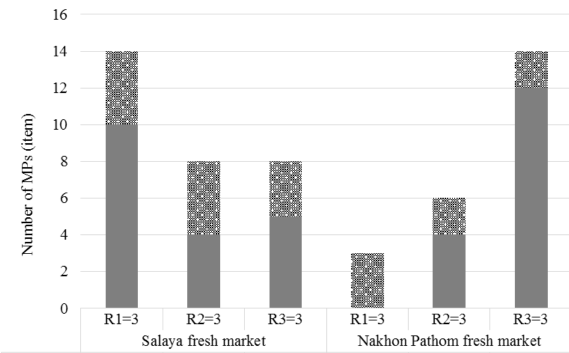
A total of 18 individual green mussel (9 individuals, 3 replicated in two fresh markets) were analyzed. All soft tissue mussel samples from the two local fresh markets contained a total of 53 microplastics, with a detection rate of 100% for each individual.

At Nakhon Pathom fresh market, the average abundance of microplastics in green mussels was 3.33±1.15 items/individual or 0.51±0.22 items/g (wet weight of soft tissue), while at Salaya fresh market, the average abundance was 2.56±1.90 items/individual or (Linnaeus, 1758) 0.30±0.22 items/g (Table 1). The bivalve green mussel *Perna viridis* is a filter feeder that absorbs microplastics in seawater while feeding. Due to the lack of data on microplastics in seawater in this study, the amount of microplastics in mussels is most likely proportional to the amount of microplastics in the water from which they are sourced. Green mussels for the two fresh markets are expected to come from the same seafood port near Bangkok. The Port's seafood market is very close to the microplastics high- value point, which may increase the likelihood of polluted local shellfish and increase microplastic abundance.

In the current study, 35% H_2O_2 was used to breakdown the soft tissue of green mussels, and many different types of microplastics, including rich fragments and fibers, were discovered (Fig. 2). When using H_2O_2, it is critical to keep the maximum weight of soft tissue under control. Too much soft tissue in one replicate usually necessitates a longer digestion time and may even necessitate digestion repetition (Mathalon & Hill, 2014). According to these results, the addition of no more than 5 g of tissue and approximately 200 mL of H_2O_2 in a 1 L glass beaker produced a good digestion effect, as reported by Li et al. (2015).

2. Microplastic characteristics

The shape, color, and size of microplastics were used to classify them (Fig. 2). The most common shape of microplastics found in mollusks was fibers (66%), followed by fragments (34%) (Fig. 2 and Fig. 3). These results supported previous findings that fibers were the most abundant shape of microplastics in oysters captured from Chinese coastal areas (Teng et al., 2019), the Bizerte lagoon (Northern Tunisia) (Abidli et al., 2019), and the northern Persian Gulf (Naji et al., 2018). Fibers, it has been proposed, are one-dimensional materials that easily degrade into smaller pieces, possibly leading to their widespread presence in marine fish stomachs, intestines, and gills (Koongolla et al., 2020). The fibers could be derived primarily from laundry wastewater. Moreover, the pervasive use and removal of fishing gear are major sources of fiber (Browne et al., 2011). De Witte et al. (2014) discovered that quayside mussels stored a high number of fibers, which could be related to port fishing activities including boat landing, net repair, and waste disposal.



	R1=3	R2=3	R3=3	R1=3	R2=3	R3=3
	Salaya fresh market			Nakhon Pathom fresh market		
Fragment	4	4	3	3	2	2
Fiber	10	4	5	0	4	12

Fig. 3 Microplastic type in edible tissue of green mussel sole in two fresh markets. R is indicated as replication in each market

Blue microplastics were the most common (62%), followed by violet (38%) (Fig. 4). The color composition varied slightly between ports, as reported by Wang et al (2021). The color of microplastic composition matched

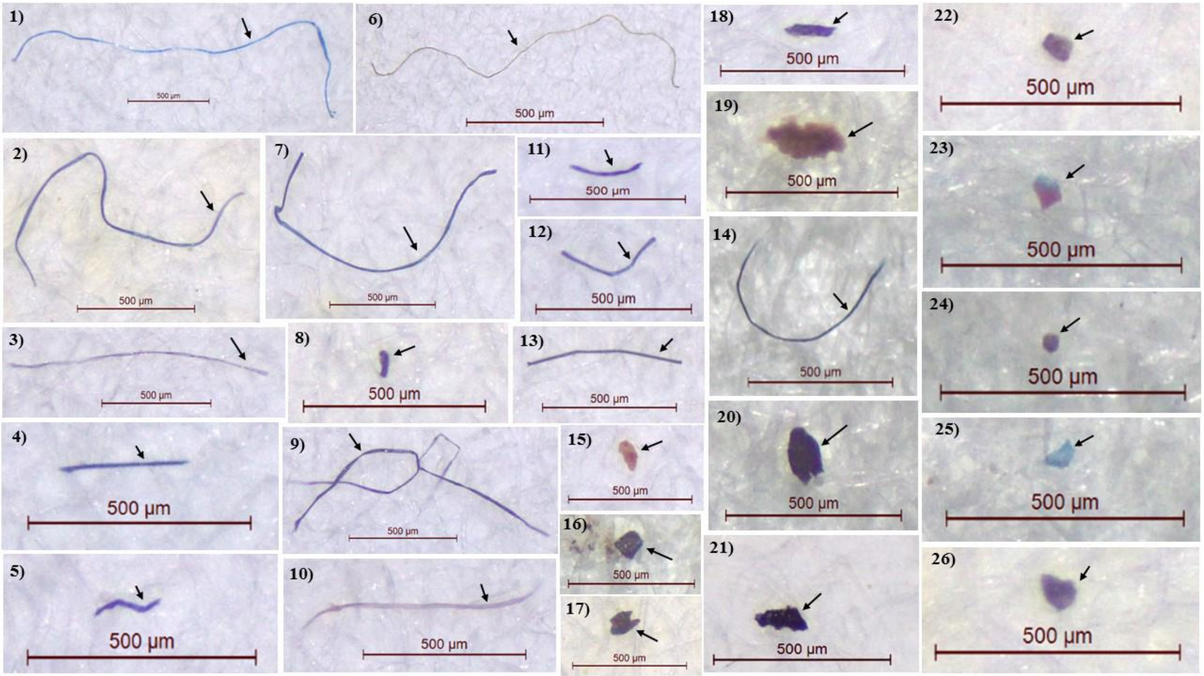


Fig. 2 Two kinds of microplastics in the filter feeding bivalve Perna viridis (1-14 = fiber; 15-16 = fragment)

that of clams collected from 21 sites (freshwater and estuarine) in the Yangtze River's middle and lower reaches (Peng et al., 2017).

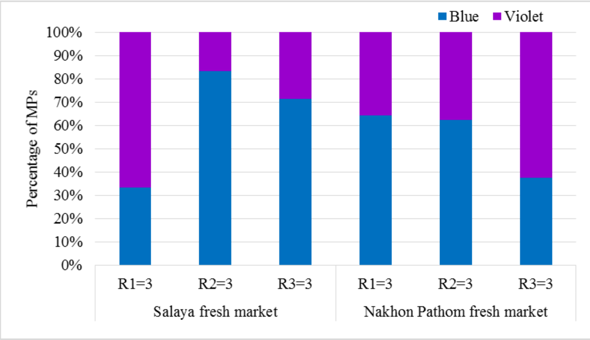


Fig. 4 Percentage of microplastic color in soft tissue of green mussel sold in two fresh markets. R is indicated as replication in each market

Particle sizes ranged from 200 to >500 μm (Fig. 4). The most common particle size range was 250-500 μm (41.6%), followed by 200-250 μm (35.8%) and large microplastics (approximately > 500 μm) (22.6%) (Fig. 5). This proportion was comparable to the amount of microplastics discovered in commercial bivalves and oysters collected from other Chinese coastal areas (Li et al., 2015; Teng et al., 2019). As particle size increased, the abundance of microplastics decreased. Among microplastics smaller than 500 μm in mussels, the size range 250-500 μm was the most abundant,

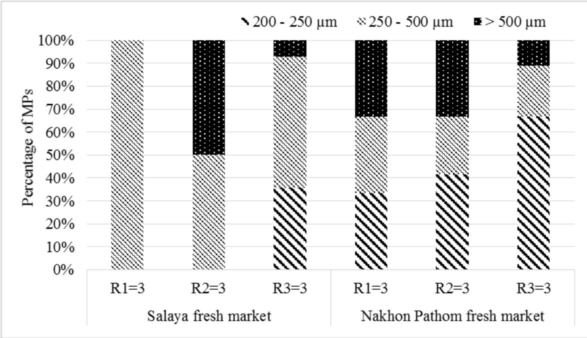


Fig. 5 Microplastic size distribution in edible tissue of green mussel sole in two fresh markets. R represents replication in each market

followed by the size range 200-250 μm (Fig. 5). Smaller particles are often more gathered in organisms than larger particles, according to various studies (Wright et al., 2013; Deng et al., 2017). Dawson et al. (2018) demonstrated that microplastics can fragment even more during digestion. This could explain why bivalves contain a higher concentration of smaller microplastics.

3. Source for microplastics

A number of 53 suspicious microplastics were chosen at random and examined using FT-IR. Mollusks contained polymers such as polyethylene terephthalate (PET), polyvinyl alcohol (PVA), and poly (propylene glycol) methacrylate (PGM) (Fig. 6). Figure 6 shows the FT-IR spectrum of each polymer. PET was the most common type of polymer. The polymer composition in this study

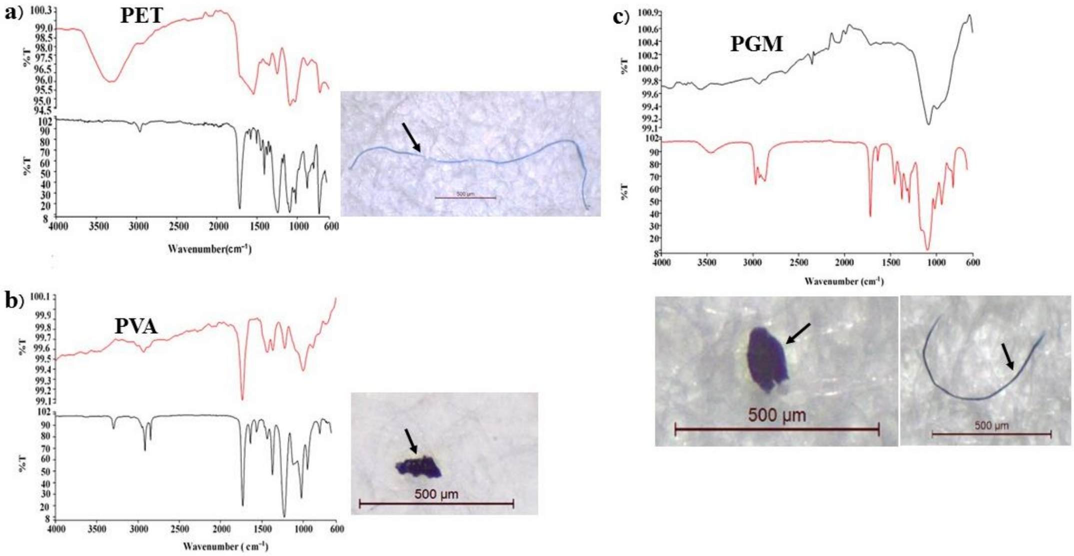


Fig. 6 FT-IR spectra of microplastics found in fresh market green mussels in Thailand. The black line represents the standard plastic spectrum, and the red line represents the microplastics in green mussels. (a) PET (Polyethylene terephthalate) (b) PVA (Polyvinyl alcohol) (c) PGM (Poly(propylene glycol) methacrylate

was dominated by polyethylene terephthalate, which was attributed to a significant proportion of the total, and PET was found in all three replicates. This finding is in accordance with microplastics found in three sessile invertebrates on Thailand's eastern coast, including *Balanus amphitrite, Saccostrea forskalii,* and *Littoraria* sp. (Thushari et al., 2017) and two bivalves (*Crassostrea gigas* and *Mytilus edulis*) on France's Atlantic coast (Phuong et al., 2017). PET is commonly used in the production of plastic bottles and food packaging bags, but it is also being used in the production of textile and garment products (Park et al., 2004). As a result, land-based sources of PET may be a significant source. PVA is used as a plastic coating in laundry and dish cleaning agents, as a sizing and finishing agent in textiles, and as a thickening or coating agent in the paper and food industries for paints, glues, meat packaging, and pharmaceuticals (DeMerlis & Schoneker, 2003).

4. Microplastic accumulation in bivalve organism world wide

Several studies have been published on the contamination of bivalves with microplastics around the world, including this one (Table 2). Because of their small size and high persistence, microplastics have the potential to be consumed by a wide range of marine organisms (Cole et al., 2011). Microplastics have also been implicated in trophic level transfer, in which higher level organisms eat other species that have already consumed microplastics, increasing the possibility of biomagnification (Farrell & Nelson, 2013).

Because of their potential impact on human health, microplastics in seafood are a source of concern. Cho et al. (2019) report that the amount of microplastics consumed by Jiangsu coastal residents through shellfish consumption is relatively low by global standards, as well as lower than the national average (6636 items/ person/ year) . The region's microplastic consumption is comparable to that of France (1139 items/person/year). Jiangsu's bivalve consumption is comparable to that of other coastal areas in China, France, and Spain. Microplastic ingestion, on the other hand, is relatively low. This is because Jiangsu bivalves have a lower concentration of microplastics. Previous research has found that the digestive tract has the highest level of microplastics (Beyer et al., 2017; Kolandhasamy et al., 2018). To decrease microplastic consumption, it is suggested that the digestive tract be removed prior to cooking mollusks (Seltenrich, 2015). Several laboratory studies have shown that microplastics or nanoplastics

Table 2 Microplastic contamination in bivalves in each location in the world

Location	Organism	Microplastic	References
French-Belgian-Dutch coastline	*Mytilus edulis*	0.2±0.3 microplastics g^{-1}	Van Cauwenberghe et al. (2015)
Santos Estuary, Sao Paulo, Brazil	*Perna perna*	75% of mussels had ingested microplastics	Santana et al. (2016)
Belgian Coastline and Netherlands	*Mytilus edulis, Mytilus galloprovincialis, Mytilus edulis/ galloprovincialis hybrids*	2.6 – 5.1 fibers 10 g^{-1}	De Witte et al. (2014)
Commercial mussel farm - Germany	*Mytilus edulis*	0.36 ± 0.07 particles g^{-1} (ww)	Van Cauwenberghe & Janssen (2014)
Commercial mussel farm - Germany	*Crassostrea gigas*	0.47 ± 0.16 particles g^{-1} (ww)	Van Cauwenberghe & Janssen (2014)
Nova Scotia – wild mussels McCormack's Beach Rainbow Haven Beach	*Mytilus edulis*	126 particles $mussel^{-1}$ 106 particles $mussel^{-1}$	Mathalon & Hill (2014)
Nova Scotia – farmed mussels	*Mytilus edulis*	178 particles $mussel^{-1}$	Mathalon & Hill (2014)
China	*Mytilus edulis*	0.9 to 4.6 items g^{-1}	Li et al. (2016)
Thailand	*Perna viridis*	0.51±0.22 items g^{-1} (ww)	This study

can enter tissues or the circulatory system, such as by passing through the gut lining or gill structures. As a result, the organism may be unable to remove these microplastics, resulting in increased accumulation and negative effects (Brennecke et al., 2015; Lu et al., 2016).

The toxicity of microplastic exposure in mussels is unknown due to a lack of data. Microplastics, according to ecotoxicology studies in marine species, may cause gut inflammation by altering intestinal permeability and dysbiosis (Qiao et al., 2019).

Conclusion

As a result of this study, microplastics were discovered in the green mussel *Perna viridis*, which was obtained from local fresh markets. Microplastic fibers and fragments were found in the mussel soft tissue. This could be reflected in the abundance of microplastics obtained from filter feeder organisms. The findings of this study contribute to greater evidence that microplastics occur in the soft tissue of bivalves. Ingestion of green mussel bivalve is a route of human exposure to microplastics because these organisms are frequently eaten whole without digestion.

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