Preliminary investigation of microplastic contamination in river snails (*Filopaludina martensi*) in Eastern Thailand and evaluation of human exposure

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Abstract. Microplastic contamination in the aquatic environment is a growing environmental issue with implications for food safety concerns. This study investigated microplastics in river snails (*Filopaludina martensi*) obtained from local markets in the eastern province of Thailand. The study analyzed the abundance, shape, size, color, and polymer type of microplastics extracted from river snail tissue and estimated human intake of microplastics through river snail consumption. The results revealed that the average microplastic abundance was 88.63 ± 51.18 particles/g wet weight. The predominant microplastics were fibers and fragments smaller than 1.0 mm, with colors ranging from transparent-white. Polymer analysis indicated that river snails primarily contained polyethylene (PE), polypropylene (PP), and polystyrene (PS). Microplastics' estimated annual intake (EAI) was significantly higher than the average in other countries. This suggests that humans may ingest microplastics through food consumption due to the biomagnification of microplastic accumulation in the food chain. Given this elevated level of human exposure to microplastics, there is an urgent need to develop policies to regulate the use, management, and disposal of plastic waste in Thailand.

1 Introduction

Microplastics, once primarily associated with environmental pollution, are gaining widespread attention due to their impact on food safety and their potential to harm human health [1]. Microplastics are considered a pathway for seafood-borne risks because they can transport various substances that threaten seafood safety, including heavy metals, persistent

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organic pollutants, microbial infections, and naturally occurring additives, pigments, and dyes found in plastics [2]. One of the most common routes for microplastics to enter the human body is ingestion. Shellfish are a significant component of the human diet. When it comes to human exposure through the consumption of aquatic species, the exposure depends on the specific parts consumed. Research indicates that most microplastics accumulate in the digestive tract and gills [3]. Since shellfish are typically consumed whole, including the gastrointestinal tract [4], contaminated shellfish containing microplastics represent a noteworthy source of human exposure.

Over the past few years, research has been conducted on microplastics in various marine mollusks, including commercially harvested, wild, and aquaculture bivalves like mussels, oysters, and cockles [5-10]. Nonetheless, there have been limited surveys that adequately cover gastropod mollusks. Previous studies have explored the connections between microplastics in water/sediment and mollusks. For instance, microplastics were found in sea snails (*Ellobium chinense*) from the mangrove forests in the northern part of Beibu Gulf, China [11]. Another study identified microplastics in Gonggong snails (*Laevistrombus turturella*) and sediment on Bintan Island, Indonesia [12]. Akindele et al. (2019) investigated microplastic pollution in the West African river system using gastropods as indicators [13]. Only a few studies, as well as independent national surveys regarding mollusk populations, have assessed the dietary intake of microplastics through the consumption of gastropod mollusks [14].

Filopaludina martensi, an herbivorous aquatic gastropod mollusk locally known as "Hoi Khom," is one of the most commonly abundant species consumed in various Southeast Asian regions, including Thailand. It is traditionally collected as a food source due to its edibility [15]. These river snails are typically hand-picked during the rainy season. Furthermore, *F. martensi* holds significant economic value as a commercial species for many river communities.

However, the available literature on microplastic contamination in shellfish in Thailand is limited. Recent research has highlighted the significant impact of microplastic pollution on coastal areas of Thailand [16-19]. Consequently, numerous studies examining microplastic contamination in marine shellfish, including green mussels (*Perna viridis*), blood cockles (*Tegillarca granosa*), clams (*Marcia optima*), and lampshells (*Lingula anatina*), have reported such contamination [8, 20]. Remarkably, despite the crucial role of rivers in microplastic transportation, there needs to be more documentation regarding microplastic contamination in freshwater shellfish. Therefore, the objectives of this research were to evaluate the prevalence and characteristics of microplastics in river snails (*F. martensi*) available in local markets in the eastern province of Thailand and to estimate the extent of microplastic ingestion resulting from the consumption of river snails by the Thai population.

2 Materials and methods

2.1 Study area and sampling

River snail (*Filopaludina martensi*) was obtained from local markets and caught by local fishermen. This study included two local markets: Mae Daeng market (MD: 12° 41' 1.428"N, 101° 16' 29.8554" E) in Rayong province and Khao Chi Chan (KC: 12° 45' 50.958" N, 100° 57' 20.0016" E) in Sattahip district located in Chon Buri province. At each local market, three retail shops were randomly selected. Each local market acquired three kilograms of river snails (2-3 cm shell length) from three different retailers. The samples were then wrapped in aluminum foil, placed in an icebox, and transported to the laboratory, where they were refrigerated at 4°C before examination. These samples were collected in December 2022.

2.2 Microplastic extraction

The microplastic extraction procedure in this study followed an established method [21]. Initially, the soft tissues of 40 individual river snails (n = 40) from each retail shop were separated. Next, samples weighing approximately 35-40 g were placed in a clean glass beaker. Organic matter was digested by immersing the samples in 69% concentrated nitric acid overnight at a ratio of 1.0 g of soft tissue to 5 ml of nitric acid. The mixture was then boiled for 2 h when no biological residue was visible. After that, the solution was diluted with warm distilled water at a 1:10 %v/v ratio. To separate the microplastics, commercial NaCl was added to create a saturated salt solution with a density of 1.2 g/cm³. After standing at room temperature for 48 h, the solution was vacuum filtered using glass fiber filter paper (Whatman GF/C, pore size 1.2 μm) to separate the microplastics. Finally, the filter membranes were stored in a clean Petri dish with adequate ventilation before further microplastic observation and identification. The same extraction methodology was applied to process samples for polymer analysis. A schematic illustration of microplastic extraction is shown in figure 1.

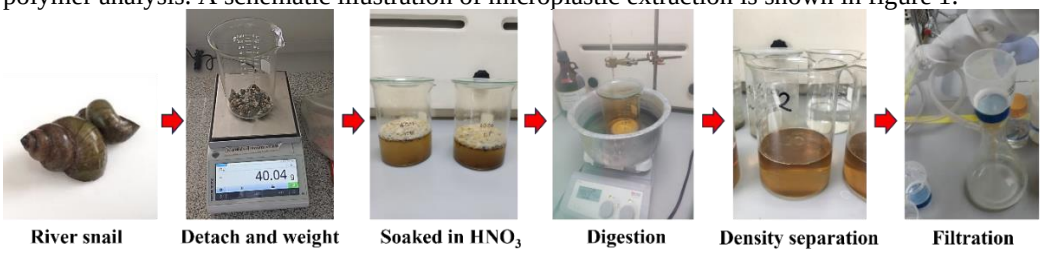

Fig. 1. Schematic illustration of microplastic extraction from river snail tissue.

2.3 Microplastic observation and identification

Using a Nikon Eclipse E200 stereo microscope from Japan, suspected microplastic particles on the filters were visually examined and photographed at magnifications ranging from 7.5x to 45x. Three types of particle shapes were identified: fibers, fragments, and films. To classify the color of these particles, the following categories were used: transparent-white (colorless/white/silver), black (black/grey), yellow (yellow/orange/brown), blue (blue/green), and red (red/pink/purple). In addition to the longest dimension, the following size categories were assessed: 1 mm, 1-2 mm, 2-3 mm, >3-4 mm, and >4-5 mm [22]. For microplastic measurement, a GF/C filter with a 47 mm diameter was divided into 1 mm² sections, and the morphology and sizes of potential microplastic particles were evaluated under a stereo microscope. Micro-Fourier transform infrared spectroscopy (μFTIR) was performed using Thermo Scientific Nicolet iS5 to identify the types of polymers in suspected microplastics. The spectra obtained were compared with the library information from the OMNIC Picta software. Based on these library comparisons, the plastic polymer can be inferred with an accuracy greater than 70% [6].

2.4 Unit and data analysis

Microplastic abundance was assessed by reporting the mean and standard deviation (SD) as the number of microplastics per soft tissue wet weight of shellfish (particles/g wet weight), making it easier to compare the abundance of microplastics with previous studies. Furthermore, the independent t-test analysis was conducted with a 95% confidence level to

compare variations in the quantity of microplastics at the sampling sites. The analysis was performed using IBM SPSS Statistics software (version 25.0).

2.5 Dietary intake of microplastics via river snail consumption

The following equation was used to calculate the dietary intake of microplastics (EAI) in river snails, which may result in the transfer of microplastics into the human body:

$$EAI = C \times IR \times 365 \quad (1)$$

where C represents the mean microplastics concentration in river snail tissues (particles/g wet weight), and the ingestion rate (IR) of river snails for consumers per capita is 0.92 g/person/day according to the National Bureau of Agricultural Commodity and Food Standards survey (Thailand).

3 Results and discussion

3.1 Microplastic abundance and characteristics

Microplastics were detected in all samples of river snails analyzed from the MD and KC markets. Microplastic abundance ranged from 29.80 to 165.50 particles/g wet weight (figure 2a). The highest number of microplastics was found in the MD market, with an average abundance of 114.93 ± 58.42 particles/g wet weight, while a lower abundance, which was two times less than that of MD, was observed in KC, with an average abundance of 62.33 ± 32.57 particles/g wet weight. The independent t-test indicated no statistically significant variations in locations (t = 1.26, p = 0.325). An average of 88.63 ± 51.18 particles/g wet weight was obtained from the sampled river snails. Microplastic abundance in river snails can be attributed to their feeding habits and the microplastic content in their environment. The river snail is a gastropod mollusk that primarily feeds on various food sources, including microalgae, filament algae, and plankton. It predominantly grazes on algae, using its radula to scrape them from rocks or the substrate's biofilm. This substrate is often dominated by fucoid algae, which can potentially trap and retain microplastics [23], making them available as part of the snails' diet. This result demonstrates how microplastics enter the river snail's system through the intermediary of the food it consumes.

River snails' tissue contains microplastics in various morphologies, including fibers, fragments, and films. Figure 2b shows that fibers (>80%) are the most common shape category, followed by fragments (2.39% to 7.10%) and film (1.80%). The diversity of microplastic shapes may have originated from the fracturing and fragmentation of large plastics due to physical processes such as photodegradation, wind, and wave action [24]. Li et al. (2016) reported that the highest occurrence of fibers in mussels' bodies, compared to other shapes, might be due to slower egestion, resulting in long-term accumulation [5]. Additionally, microfibers can be twisted or woven into food, increasing the likelihood that an organism will consume them [7]. Similar results demonstrating the dominance of microfibers have been reported in mussels [9, 25], cockles [8], oysters [7], and marine periwinkles [26, 27].

According to figure 2c, the primary size of microplastics in river snails was 1.0 mm (90%), followed by 1-2 mm (9%), with the least standard size being 2-4 mm (1%). The small-sized microplastics (< 1.0 mm) have been demonstrated in snails in many other studies [11, 28].

This result indicates that river snails selectively consume microplastics within specific size ranges. A previous investigation found that mussels preferred to ingest microplastics ranging from 7-35 µm from the water [29]. Smaller microplastics (less than 1000 µm) are comparable in size to food particles such as diatoms, leading to size selectivity in shellfish for their food intake. Furthermore, smaller microplastics may absorb heavy metals and persistent organic pollutants from the environment, posing a threat to biota when ingested [30].

The colors of microplastics isolated from the river snail tissue were identified and are shown in figure 2d. Transparent-white (>80%) was the predominant color of microplastics in river snails, followed by black, blue (comparable to red), and yellow, with averages of 9.33%, 2.26%, 2.20%, and 1%, respectively. The high proportion of transparent-white microplastics reported in our study was similar to those found in China's sea snails (*Ellobium chinense*) in China [11]. This result contrasts with the color patterns of microplastics in bivalves, which exhibit a high proportion of dark colors (blue and black) [6, 7]. Since bivalves are filter feeders, color preference may not significantly influence the uptake of microplastics. In contrast to herbivorous river snails, microplastics can be indirectly absorbed by ingesting food that already contains microplastic particles.

Regarding the polymer types, three types of low-density polymers were identified in river snail tissue: polypropylene (PP), polyethylene (PE), and polystyrene (PS). The most dominant polymer type was PE (30%), followed by PP (20%) and PS (20%) (figure 3a). Given Thailand's production and consumption patterns, the PE, PP, and PS abundance seems reasonable. PP is used in clothing, blankets, fiber-based products, food packaging, and chemical containers, while PS is used in foam products, plastic cups, protective packaging, bottles, and food containers. PE is a raw material used in bottles, homeware, pipes, and toys. Consequently, these polymer types are commonly found in the aquatic environment. Additionally, this analysis found evidence of a non-polymer natural fabric, specifically linen. Under a stereo microscope, it can be difficult to distinguish these non-polymer objects from polymers, and they can be mistaken for microplastics. The FTIR spectra of representative microplastics from the river snails, along with the shapes of selected microplastics under a µFTIR microscope, are shown in figure 3b.

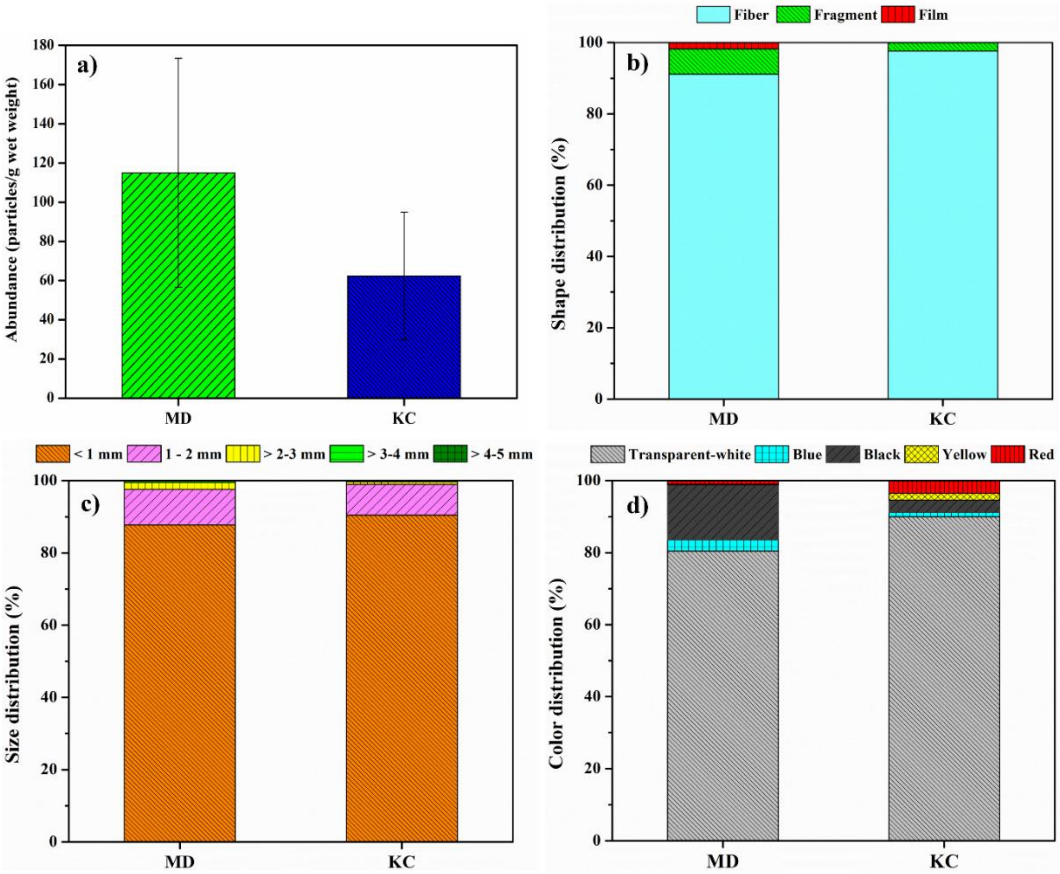

Fig.2. Abundance of microplastics in river snails (*F. martensi*) in the eastern province of Thailand (a), illustrating microplastic distribution by shape (b), size (c), and color (d).

3.2 Human microplastic exposure via river snail consumption

We assessed the Thai population's annual per capita intake (EAI) of microplastics through river snail consumption using Thai food consumption statistics and microplastic concentrations discovered in river snails. The EAI through the consumption of river snails was 29,761.95 particles/person/year. To compare the EAI values with this study, we used shellfish such as mussels, cockles, clams, and oysters, which are consumed whole. As shown in Table 1, the current result is 2.5 to 2.7 times higher than those found in Italy (11,970 particles/person/year) [4], and Germany (11,000 particles/person/year) [31]. The intake of microplastics by the population of Spain [14], Ghana [6], Canada, and South Korea [4] was found to be 8,407, 8,103, 754-4,160, and 243-1,182 particles/person/year, respectively, which is between 3.5 and 122.5 times lower than that of this study.

Our findings suggest that the annual dietary exposure for Thai river snail consumers might be significantly higher, and we advise people to avoid consuming river snails frequently. Although we observed a significant level of human exposure in this study, there are still uncertainties to address. It remains uncertain whether the quantity of ingested microplastics corresponds to the amount absorbed and whether cooking substantially impacts plastic toxicity. While the research offers crucial foundational information regarding the presence

and quantity of microplastics in river snails from Thailand's freshwater sources, we acknowledge the limitation of a relatively small sample size. Therefore, we recommend further studies on a larger scale.

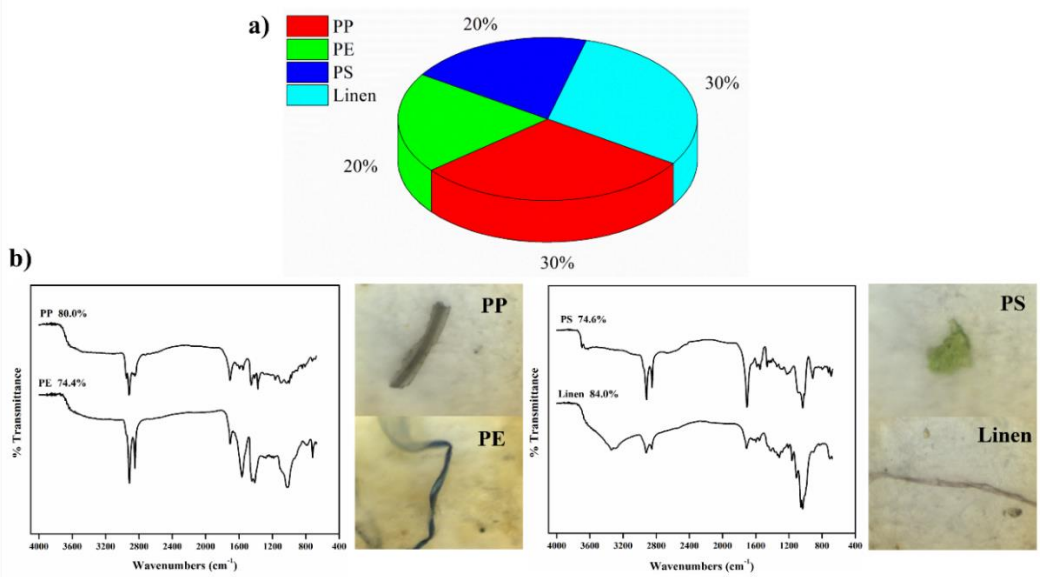

Fig.3. Polymer type distribution (a), and FTIR spectra of representative microplastics from the river snails, along with the shape of selected microplastics under a microscope of µFTIR (b).

Table 1. Estimations of the annual consumption of microplastics through the ingestion of shellfish across various countries worldwide.

Country	Species	Annual microplastic intake (EAI) (particles/person/year)	Reference
Thailand (Eastern region)	River snail (*F. martensi)*	29,761.95	This study
Italy	Mussel	11,970	[4]
Germany	Mussel (*Mytilus edulis*)	1,800 - 11,000	[31]
Canada	Mussel	8,407	[4]
Spain (North-west Mediterranean Sea)	Mollusks (clams, mussel, oysters)	8,103	[14]
Ghana (Gulf of Guinea)	Oyster (*Crassostrea tulipa*)	754 - 4,160	[6]
South Korea	Oyster (*Crassostrea gigas*) Mussel (*Mytilus edulis*) Clam (*Tapes philippinarum*) Scallop (*Patinopecten yessoensis*)	243 - 1,182	[4]

4 Conclusions

This study aimed to examine the occurrence of microplastics in river snails purchased from local markets in Thailand's eastern province and assess the likelihood of human exposure to them. The samples of river snails contained an average of 114.93 ± 58.42 particles/g wet weight. Microplastics primarily appeared in the form of fibers and were transparent-white in color. Most microplastics were smaller than 1.0 mm in size. The sample contained three different types of polymers, including PE, PP, and PS, as well as natural fibers produced from linen. The estimated annual intake (EAI) for river snails was 29,761.95 particles/person/year. This value is significantly higher than the global mean EAI for consuming shellfish. These findings suggest that individuals should consider limiting their consumption of shellfish, especially river snails.

We would like to express our gratitude to the Scientific Instrument and Analytical Testing Center (SIATC) for providing the equipment. This research was supported by the Faculty of Science, Energy, and Environment, King Mongkut's University of Technology North Bangkok (Rayong Campus), Thailand.

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