The Contamination of Microplastics Ingested by Freshwater Molluscs in the Mun River, Thailand

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Abstract
This study aims to determine the amount of microplastic contamination in freshwater molluscs consumed in the Mun River. The predominant species of freshwater molluscs included Filopaludina martensi, Mekongia sphaericula, Scabies crispate, and Corbicula moreletiana. A total of 48 samples were obtained from each species, including four distinct areas characterized by varying land uses. The mean differences in microplastics were estimated using multiple linear regression. The results showed that the average of microplastics contamination in freshwater mollousecs soft tissue was 11.19±6.89 pieces/individual. The microplastic contaminants in Scabies crispate was significantly higher than those of other species. The level of microplastic contamination in the studied mollousecs species were Scabies crispate > Corbicula moreletiana > Mekongia sphaericula > Filopaludina martensi, which Scabies crispate is more than that of the Filopaludina martensi 5.72 pieces/individual (95% CI: 4.25-7.20, p < 0.001). The average microplastic contamination in molluscs from urban areas was significantly higher than that in other areas. The studied area was urban area > confluence of Mun and Chi River area > agricultural area > natural area, which the urban area is more than that of the natural area with 18.41 pieces/individual (95% CI: 16.94-19.89, p <0.001). And the most common polymer type of microplastic was Polypropylene (PP), most common shape filaments/fiber and was blue most common color. The interpretation of this observation is that the consumption of different types of freshwater molluscs, especially bivalve and sampling sites especially in the urban area may affect microplastics exposure.

Keywords: Aquatic Animal, Freshwater Molluscs, Microplastic Contamination, The Mun River, Urban Area

1. Introduction

The consumption of plastics currently poses a risk to the environment since it produces microplastic particles (less than 5 mm in size). Improper disposal of commercial, industrial, and residential waste is the main cause of microplastic contamination in freshwater ecosystems [1]. There is concern about the possible negative effects on ecosystems and species due to its ability to absorb microplastics and gather other pollutants, such as heavy metals [2]. pesticides [3]. Currently, microplastic contamination has been found to have highly contaminated the aquatic environment. As a result, microplastics can reach every level of the food chain. Therefore, many countries in the world have studied the contamination of microplastics in water ecosystem, seawater [4] and [5], and freshwater [6] and [7], especially, microplastic accumulation in aquatic animals [8][9] and [10], which are aquatic creatures that are widely consumed by humans. Especially molluscs, which are part of the benthic community, that act as a bioindicator of microplastic pollution [11], as the potential human health risk posed by microplastics in molluscs were at high levels [12]. It suggests that the consumption of molluscs is an important route of microplastics exposure [13]. Microplastic accumulation in the molluscs can cause health effects in humans from the chemical in microplastic.

In Thailand, contamination has been reported through microplastic accumulation in freshwater ecosystems such as water, sediment [2][14] and [15], aquatic animals that are popular for local consumption, such as edible arthropods [16], shrimp [17], fish [18] and [19], molluscs [18] and [20]. The Mun River is situated in northeastern Thailand and is one of the largest tributaries of the Mekong River in Thailand. It is considered the main water source for agriculture, aquaculture, fish farming, fishing activity, water supply, and sewage from industrial factories and residential areas. As it flows through five provinces before entering the Mekong River at Ubon Ratchathani Province. The Mun River is an area that is repeatedly flooded due to damage from both the Chi River and the upper Mun River, Especially in Ubon Ratchathani Province, it's a floodway. As a result, floods affect it almost every year. Heavy rains and flooding can carry large amounts of heavy metal debris and pesticides into waterways [21], heavy rains and severe floods can dramatically increase microplastic levels [22]. These pollution sources may also be the source of microplastics in the Mun River.

Therefore, the objective of this study is to ascertain the occurrence of microplastic contamination in freshwater molluscs found in the Mun River, which is the type popularly consumed by the community. This research is significant as Thailand has been actively investigating microplastics in freshwater environments, but a lack of previous research has examined the bioaccumulation of microplastics in freshwater animals, specifically in the Mun River.

2. Materials and Methods

2.1 Sampling Sites

A study was conducted on freshwater mollusc samples obtained from four sampling stations along the Mun River in the northeast of Thailand, which were subjected to varied land uses, selected in the lower part of the river, extending from the upper section to the lower section, inside Ubon Ratchathani Province. The four sampling locations represented distinct geographical regions (Figure 1). The first spot of interest indicates the highest point of the investigation where the Mun and Chi Rivers converge, before passing through the city of Ubon Ratchathani (15°10′54.6″N 104°42′55.9″E). The second site is an agricultural area (15°13′18.2″N 104°45′50.4″E), the third site is an urban area (15°13′23.0″ N 104°51′24.5″ E), and the fourth site is a natural area (15°16′40.7″N 104°59′53.8″E).

2.2 Sample Collection and Preparation

2.2.1 Sample collection

Local fishermen collected freshwater molluscs during the dry season (March – May 2023) using 100x100 centimeter frames at a depth of 15 centimeters. The collected samples were then sorted by sifting them through a 2x2 mm sieve [14]. Subsequently, the freshwater molluscs were categorized based on their weight and measurement (Table 1). The freshwater molluscs samples were stored at a temperature of -20°C before being examined for microplastics. The first location marks the highest point of the investigation where the Mun and Chi Rivers converge, before to flowing through the city of Ubon Ratchathani.

2.2.2 Sample preparation

Frozen freshwater molluscs samples were thawed for 15 minutes at room temperature and rinsed with deionized water prior to separating the soft tissue from the molluscs. The fresh weight of the soft tissue of molluscs was recorded.

2.2.3 Microplastic separation

After dissection, the soft tissue of molluscs was placed in 500 ml Erlenmeyer flasks, and 30% H_2O_2 was used to digest the organic matter [23]. The volume of H_2O_2 was based on the weight of the soft tissue (200 ml/ soft tissue 5 grams). The conical flask was covered with aluminum foil. Extracted samples were placed in an incubator shacking (80 rpm) at 85°C for 24 hours by using an incubator shacking (LT-x Lab Therm-kunner) to digest the organic matter. After breaking down the organic matter, it was left at room temperature, filtered, and added to the flasks.

Table 1: Descriptions of microplastic contamination

Species	Feeding	Length (cm) Mean ± S.D.	Microplastic contamination	
			N	%
F. martensi	grazer	3.13±0.52	47	97.91
M. sphaericula	grazer	3.29±0.96	47	97.91
S. crispate	filter	4.17±0.31	48	100.00
C. moreletiana	filter	2.82±0.62	48	100.00
Total			190	98.95

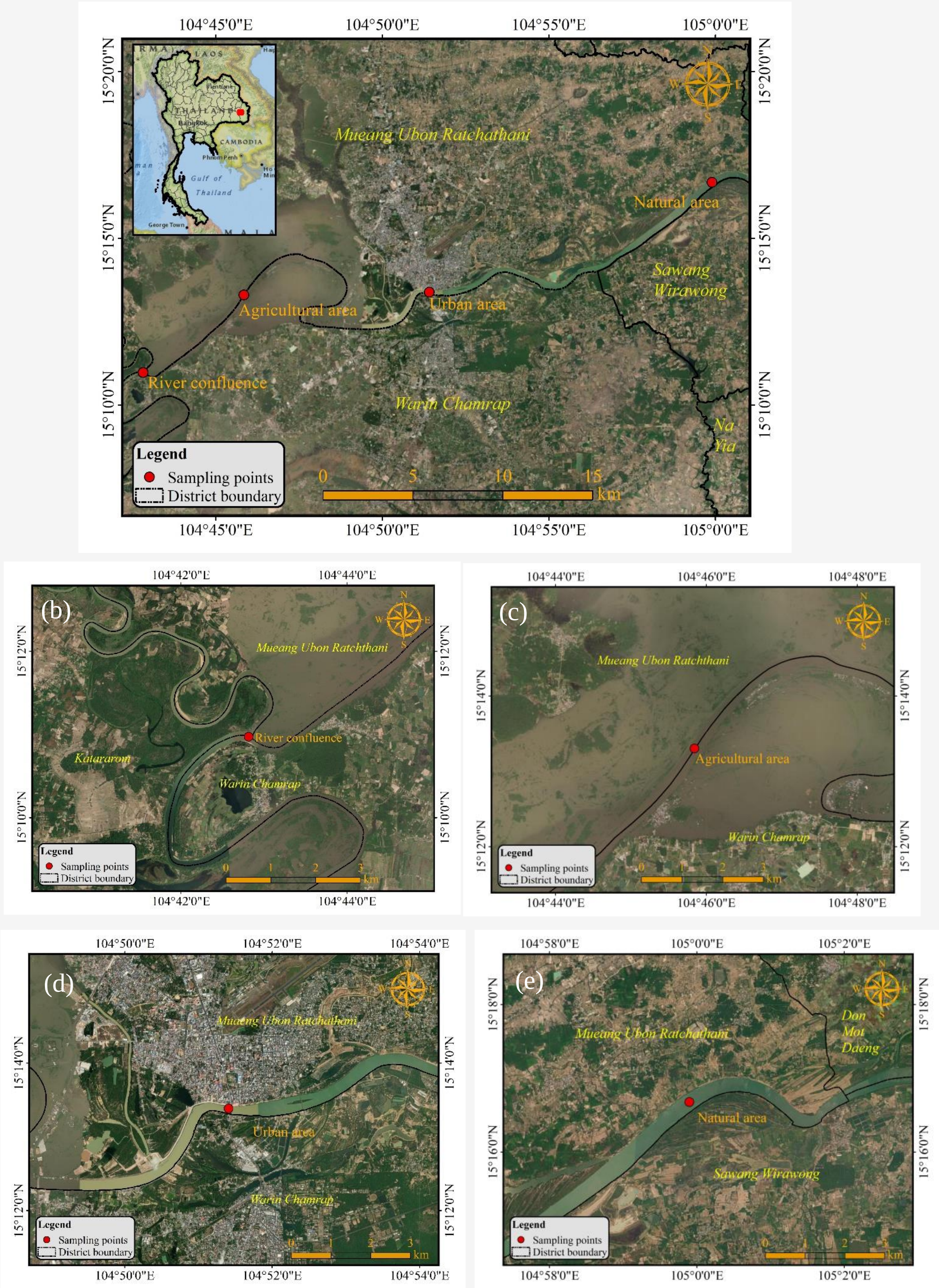

Figure 1: Data collection locations (a) sampling points along the Mun river in Ubon Ratchathani province (b) river confluence (c) agricultural area (d) urban area (e) natural area

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A saturated NaCl solution (about 300 g/L) was then added to the flasks to separate the microplastics [24]. The microplastic density was separated by shaking for two minutes. Then, the supernatant was pipetted and filtered through a glass microfiber filter (Whatman GF/C 0.47 mm pore size). Then, the filter paper was dried at 50°C for 24 hours.

2.3 Inspection of Microplastics and Identification

The filter paper was placed on petri dishes to record the numbers of microplastic particles, size, shape, colors, physical characteristics, and measured for their longest dimension, divided under a stereomicroscope (FULIFILM X70). The microplastic polymer type was examined by Fourier transform infrared spectroscopy (FT-IR) (Thermo Scientific: nicolet 6700).

2.4 Statistical Analysis

Descriptive statistics, percentage, mean, standard deviation, and inferential statistics were obtained by using the Stata program, version 14. Analytical statistics were used to compare the differences in microplastic content among molluscs across different sampling sites, species, and river banks (districts), 95% confidence interval (95% CI), and P-value using multiple linear regression.

3. Results and Discussion

3.1 Microplastic Contamination

Microplastics were found in all species of molluscs collected from the Mun River in four areas. A total of 192 individual freshwater molluscs were identified into four species (Figure 2). We observed microplastics inside the soft tissue of 190 individuals (98.95% of total molluscs samples). The percentage occurrence of microplastics in each species ranged between 97.91 and 100.00%, with the highest in bivalve were *Scabies crispate* (100%) and *Corbicula moreletiana* (100%) (Table 1).

The average of microplastic in molluscs' samples at the study area is 11.16±7.84 pieces/individual, the highest numbers of microplastic are found at the urban area is 21.52±4.75 pieces/individual, whereas the lowest numbers of microplastic is found at the natural area is 3.20±1.30 pieces/individua (Figure 3), when comparing the average number of microplastics at the urban area with the natural area, it was found that the urban area is more than that of the natural area 18.41 pieces/individual (95% CI: 16.94-19.89, $p < 0.001$) (Table 2), consistent with numbers of microplastic that were found in sediment highest at the urban area, lowest at the natural area and these areas were a residential area and a dense commercial area along the river, including houses, raft houses, restaurants, fresh markets, food markets along the Mun River, walking street market along the Mun River, close to the discharge point of the wastewater treatment system. This caused the molluscs living in this area to swallow microplastics contamination in their natural food. Molluscs can therefore accumulate similar amounts of toxins and microplastics in sediments and water [25]. In addition, the proportion of ingested microplastics in this study was higher than in Italy (0.92 ± 1.21 pieces /individual) [26], and China (0.4-5.0 pieces/individual) [27]. The microplastic accumulation in mollusces at Ubon Ratchathani distric river bank was 11.19±7.88 pieces /individual more than that in mollusces at Warin Chamrap district river bank (95% CI: -0.38-1.99, $p = 0.183$). However, there was no significant difference.

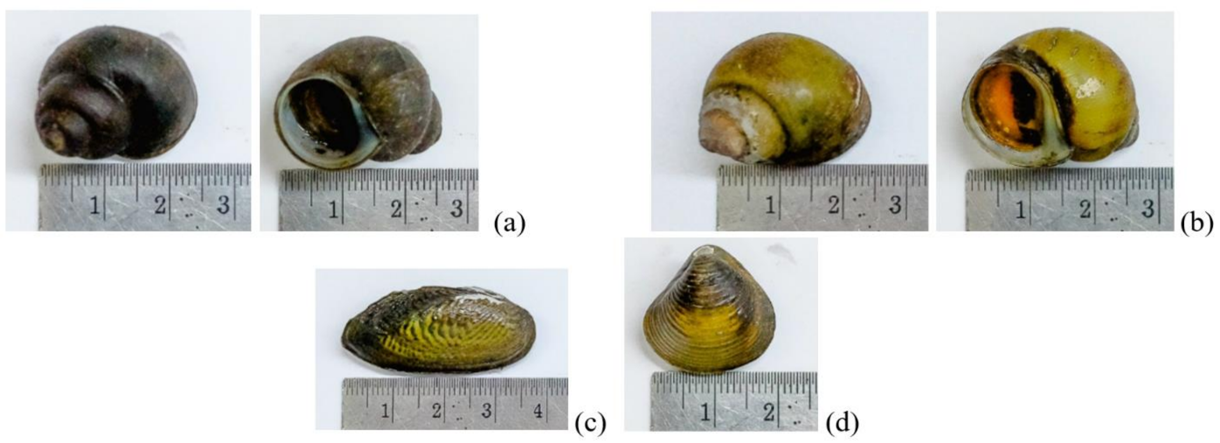

Figure 2: Molluscs sampled in the Mun River (Thailand), (a) *Filopaludina martensi*, (b) *Mekongia sphaericula,* (c) *Scabies crispate*, (d) *Corbicula moreletiana*

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Table 2: Comparison average of microplastic contamination in molluscs separated by sampling site and species

Variable	Microplastic (piece)	$\bar{X}$ ± S.D.	Mean Difference	95% CI	P-value
Sampling sites					
Natural area	154	3.20±1.30	Ref.	Ref.	
Agricultural area	430	8.95±3.20	5.75	4.27-7.22	0.049
Confluence of Mun and Chi River area	527	10.97±6.03	7.77	6.29-9.24	0.006
Urban area	1,033	21.52±4.75	18.41	16.94-19.89	<0.001
Species					
Filopaludina martensi	426	8.87±5.86	Ref.	Ref.	
Mekongia sphaericula	498	10.37±7.57	1.47	0.004-2.95	<0.001
Corbicula moreletiana	525	10.93±6.89	2.06	0.58-3.53	<0.001
Scabies crispate	695	14.47±9.67	5.72	4.25-7.20	<0.001
River bank (district)					
Warin Chamrap	1,546	11.76±8.42	Ref.	Ref.	
Ubon Ratchathani	2,149	11.19±7.88	0.80	-0.38-1.99	0.183

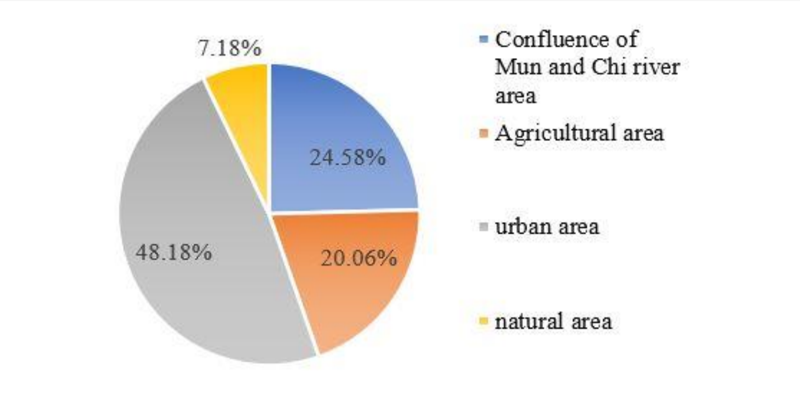

Figure 3: The Percentages of microplastic contamination by molluscs (pieces/individual) in sampling sites

The highest number of microplastics found in *Scabies crispate* is 14.47±9.67 pieces/individual, whereas the lowest number of microplastics found in *Filopaludina martensi* is 8.87±5.86 pieces/individual (Figure 4). When comparing the average number of microplastics in *Scabies crispate* (Bivalves) with *Filopaludina martensi* (Snail), it was found that the *Scabies crispate* is more than that of *Filopaludina martensi* 5.72 pieces/individual (95% CI: 4.25-7.20, p <0.001) (Table 2), bivalves are significantly more contaminated with microplastics than snails [13].

Bivalves, which are typical filter feeders that constantly filter out microbes and organic matter from the surrounding water [28], can filter out microplastics from the surrounding depositional environment [27] and [29]. Bivalves are at high risk of microplastic contamination due to their filter-feeding mechanism [9] [30] and [31]. As a result, numerous studies favour using bivalves as indicators of microplastic contamination in the marine environment [32] and [33], The ingestion of microplastics by molluscs poses a potential health risk for humans through consumption.

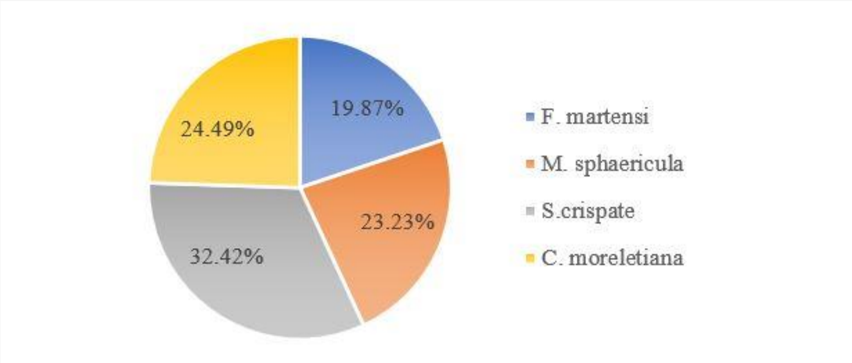

Figure 4: The Percentages of microplastic contamination by molluscs (pieces/individual) in each species

Table 3: Average size of microplastics by molluscs in each species

Species	Size of Microplastics (µm)		
	$\bar{X}$ ± S.D.	Minimum	Maximum
F. martensi	477.71±442.56	18	3,650
M. sphaericula	567.06±778.052	23	11,257
S. crispate	385.22±486.78	12	5,635
C. moreletiana	443.53±599.25	12	8,432

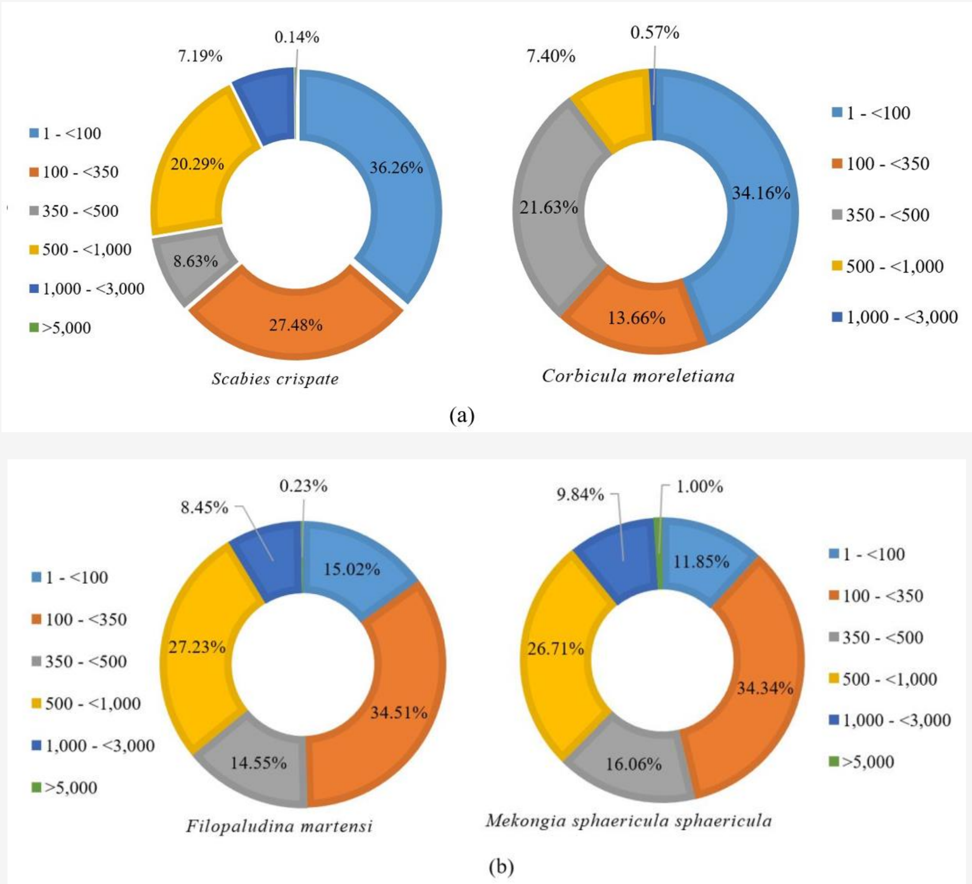

Figure 5: Percentages of microplastic contamination based on size range:(a) bivalve and (b) Snail

3.2 Size, Shape and Color of Microplastics

The average size of microplastics that had contamination in snails such as *Filopaludina martensi* and *Mekongia sphaericula* (477.71±442.56 and 567.06±778.0525 µm) was bigger than that in bivalve such as *Scabies crispate* and *Corbicula moreletiana* (385.22±486.78 and 443.53±599.25 µm), respectively (Table 3). The average size of microplastics in the snails was similar to the microplastics accumulated in snails found in the Nam Pong River [18]. Microplastic size of 1 to < 100 µm are the predominant size in bivalves, where bivalves incorporate smaller particles into their tissues (17–88 µm) because they are easier to digest, particle above this size are likely to be rejected and eliminated as feces or pseudofeces [34], while the microplastic range (100 to < 350 µm) is the predominant size of snails (Figure 5).

Fiber shape was a major type ingested by molluscs (Figure 6) [18] [10] and [27] and related to human activities [35]. An important source of fiber microplastics is sewage from washing clothes [36] and [37], and degradation of fishing gear [38]. Ten colors of microplastics were found in the soft tissue of molluscs: blue, transparent, white, red, black, purple, orange, pink, brown, and green. The three most common colors were blue, purple, and pink. This result is consistent with the results of studies on the Buriganga River in Bangladesh, where blue was the dominant color [8]. The polymer types of microplastics found in this study were studied using Fourier transform infrared (FT-IR) spectroscopy. We found a total of seven types; the most common types of microplastic polymers were polypropylene (PP), polyethylene (PE), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polystyrene (PS), nylon, and polycarbonate (PC), respectively (Figure 7).

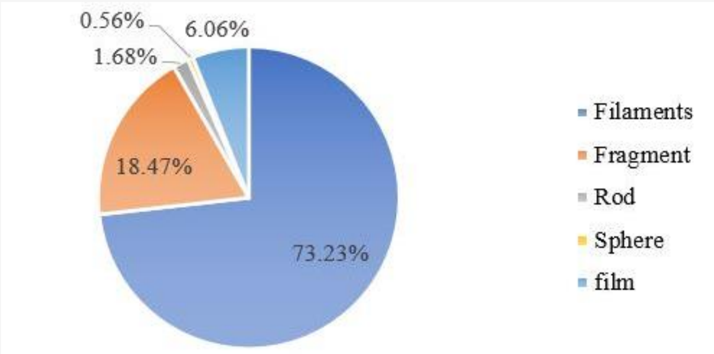

Figure 6: The percentages of microplastic contamination by freshwater molluscs of shape

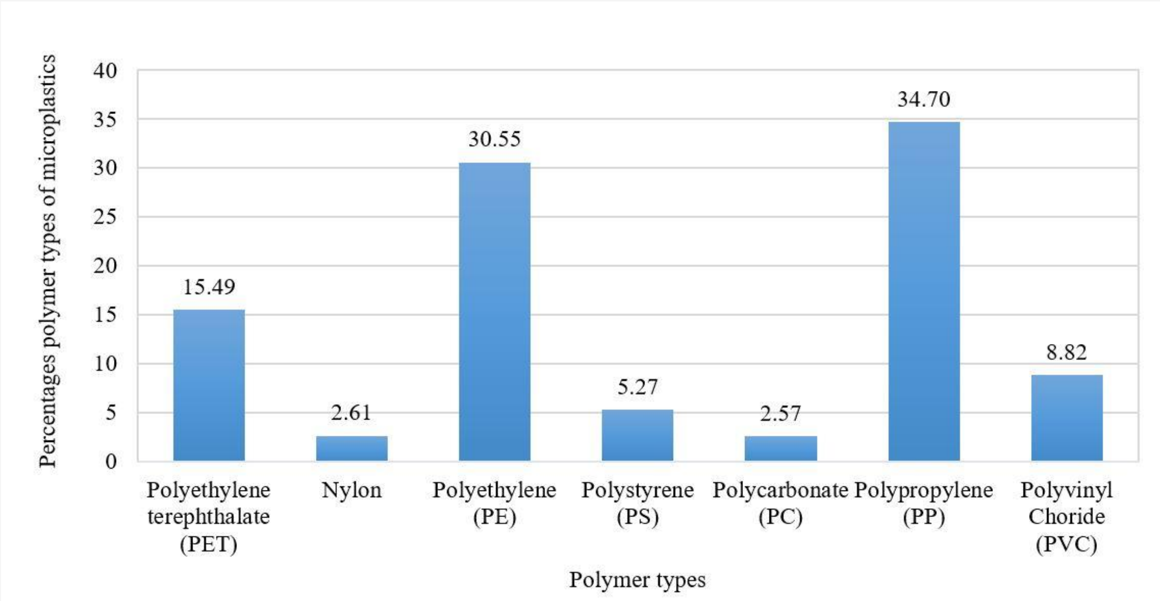

Figure 7: Percentages of polymer types of microplastics in freshwater molluscs

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This is consistent with microplastics in different fish and shellfish species in the mangrove estuary of Bangladesh, where polypropylene and polyethylene were found in the muscle tissue of benthic animals [39], and the most common polymer plastics in bivalves were mainly polypropylene [10]. PP is frequently utilized in fishing nets and lines, life jackets, ropes, mooring, and towlines [40]. Consequently, an abundance of PP and PET fibers might endorse the proposition that laundry wastewater is a significant source of microplastics [41].

4. Conclusion

The presence of microplastic contamination in freshwater molluscs in the Mun River revealed that bivalve molluscs were more heavily contaminated than snails. Additionally, metropolitan regions exhibited the greatest levels of microplastic contamination. Hence, more research is needed to investigate the level of microplastic exposure resulting from the intake of freshwater molluscs in the Mun River, the presence of toxic compounds inside microplastics, and the factors linked to the health hazards associated with consuming molluscs from freshwater sources.

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