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The accumulation of microplastics in fish freshwater in the Mun river, Thailand

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ABSTRACT

Background: Microplastic pollution mainly occurs from terrestrial sources, but research on plastic contamination in aquatic environments is limited. Freshwater fish are widely consumed throughout Southeast Asia. Microplastic contamination is becoming an important issue that leads to human health risks from consumption.

Objectives: The study aimed to determine the accumulation of microplastics ingested by fish in freshwater in the Mun River.

Methods: Freshwater fishes in the Mun River caught by local fishermen and the most dominant species consumed in the Mun River include *Paralaubuca typus*, *Phalacronotus bleekeri*, and *Morulius chrysophekaion*. Twenty-four samples from each species were collected. Microplastics in these species were analysed for abundance, size, shape, color, and type of polymer.

Results: The results showed that the average amounts of microplastic contamination in the gastrointestinal tracts (GIT) and gills of *Morulius chrysophekadion* were 17.70±8.20 pieces/fish, *Phalacronotus bleekeri* were 11.83±8.74 pieces/fish, and *Paralaubuca typus* were 10.25±7.56 pieces/fish, respectively. The average number of microplastics found in the urban area was 26.50±4.21 pieces/fish; in the agricultural area, it was 9.77±4.10 pieces/ fish; in the confluence of the Mun and Chi River areas, it was 9.50±3.51 pieces/ fish; and the natural area had 7.27±4.15 pieces/ fish, respectively. The average size of microplastics found in fish was 878.29±904.41 μm. The most common polymer type microplastic was polyethylene (PE) where the most common shape was fiber-shaped, and the most common colour was blue.

Conclusion: The study findings indicate a prevalent form of microplastic accumulation in three fish species especially among *Morulius chrysophekaion*. And findings show that every fish species in the urban area have the most contamination. As a result, fish GIT and gills should be removed before consumption to reduce exposure to microplastics instead of consuming them.

Keyword: Contamination, Freshwater fish, Microplastics, Mun River

1. Introduction

Thailand produced approximately 2.76 million tons of single-use plastic waste in 2021, accounting for 11 percent of the country's total waste output. Separation and reuse exist. The remaining 78 percent of the refuse will be disposed of as general waste. The remaining 3 percent is left unmanaged and, in the environment [1]. Plastic waste causes environmental pollution, especially small plastic or microplastic items with particle sizes <5 mm [1].

Numerous nations around the globe have initiated research on the contamination of microplastic in their environments, such as in surface waters [2], and sediments [3]. Additionally, it was discovered that contamination can infiltrate the food chain at all levels [4] especially aquatic animals' food chain [5]. There has been microplastic contamination discovered in Thailand. It has been discovered in sediments in the lower Gulf of Thailand [6], in water and sediments in the Chao Phraya River [7], and in numerous aquatic animals, such as molluscs at Chaolao and Kungwiman beaches in Chanthaburi province [8], and in fish from the upper Gulf of Thailand [9]. It is also found in freshwater aquatic animals such as fish in Bueng Boraphet [10], fish and snail in Nam Pong River [11], and fish in Chi River [12]. Microplastics smaller than 150 μm can translocate across the human gastrointestinal tract into the lymphatic system [13], leading to health issues [14]. The health effects depend on long-term exposure to microplastics via a variety of exposure routes [15].

The Mun River is a major river in the Northeast of Thailand with a length of about 630 km. Before entering the Mekong River at Ubon Ratchathani Province, it passes through five provinces. According to the report of the Environmental and Pollution Control Office No. 12 (2023), the water quality in the river has decreased significantly in the past three years (2020–2022) due to the release of household wastewater and runoff from agriculture, industry, and cities. It is also an area that is repeatedly flooded due to runoff from the upper Chi and Mun rivers [16]. Microplastic levels are found to be higher after floods [17]. These pollutions could be the sources of microplastics in aquatic animals living in the Mun River.

Therefore, it is necessary to study the accumulation of microplastics in fish in the Mun River. This will provide information to environmental regulatory agencies to develop guidelines for reducing microplastic contamination in the Mun River. Additionally, the results of the study can be used to develop guidelines for reducing human exposure to microplastics through fish consumption.

2. Methods

2.1 Study Area

This study examined the accumulation of microplastics ingested by fish in the Mun River in Ubon Ratchathani Province, Warin Chamrap District, and Mueang Ubon Ratchathani District (Figure 1). Fish samples were collected at four sampling sites in the Mun River. Among the dominant fish species, three most consumed fish species were collected as samples; such as *Paralaubuca typus*, *Phalacronotus bleekeri*, and *Morulius chrysophekadion*. Two samples of each species were collected continuously three times, totalling 72 samples. (Figure 2)

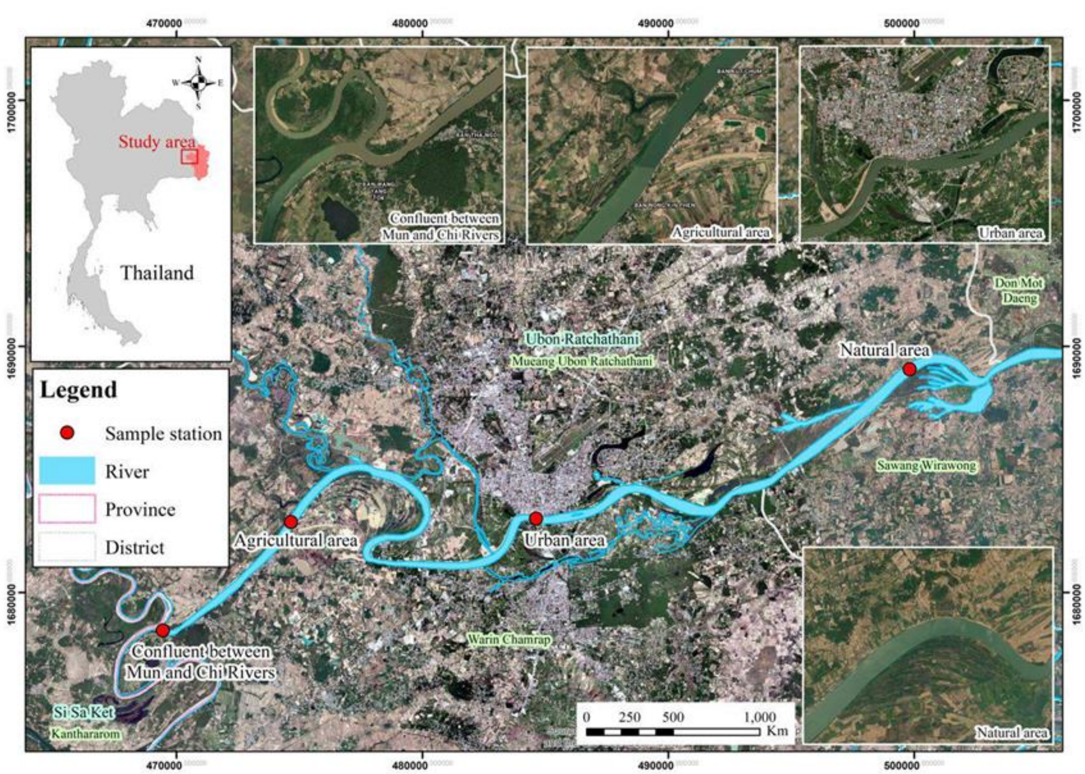
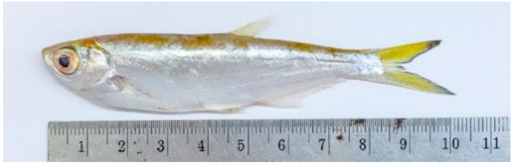


Figure 1: Sampling sites



Paralaubuca typus

Phalacronotus bleekeri

Morulius chrysophekadion

Figure 2: Fish sampled in the Mun River (Thailand)

2.2 Study Design

This was a cross-sectional descriptive study. The accumulation of microplastics ingested by fish in the Mun River were analysed for size, shape, color, and polymer type of microplastics.

2.3 Sample size and sampling

Fish were caught by local fishermen using gill nets and trawling during the dry season (February and March 2023), and the body weight in grams and length in centimetres of the fish were then recorded and wrapped in foil. The samples were then carefully transported in insulated boxes at 4°C and stored at −20 °C until further analysis.

2.4 Data Collection

We brought the frozen fishes to ambient temperature and cleaned them with distilled water. We then, separated the portions of the digestive system and intestines (the gastrointestinal tract, or GIT), and the gill. The GIT weight and gill weight (grams) were recorded. The GIT was transferred to 100-ml Erlenmeyer flasks and 30% Hydrogen Peroxide (H_2O_2) was used to digest the organic matter [18]. The volume of H_2O_2 was based on the weight of the GIT samples (approximately 30 ml/sample). Extracted samples were placed in an incubator shackled at 80°C for 24 hrs. Saturated sodium chloride (NaCl) (approximately 300 g/L) was added to the filter. The microplastic density was separated by shaking for two minutes using a shaker. The precipitate and the solution were separated into layers. Following this, the supernatant was pipetted and filtered through a glass microfiber filter (Whatman GF/C 0.45

µm pore size). After that, the filter paper was dried at 50°C for four hours. The samples were analysed for microplastics. The filter paper was placed on Petri dishes to record the numbers of microplastic particles, colour, and shapes under a stereomicroscope. Microplastics were measured for their longest dimension.

2.5 Data Analysis

Descriptive statistics were calculated and have been described as percentage, mean, and standard deviation. Data analysis was done by the STATA program, version 14.

2.6 Ethical Clearance

This study was approved by Khon Kaen University Ethics Committee for Human Research based on the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines (HE 652162).

3. Results

3.1 The abundance of contamination in fish

In this study of microplastic contamination in three fish species, *Paralaubuca typus*, *Phalacronotus bleekeri, and Morulius chrysophekaion*, 24 samples of each species were examined for a total of 72 samples. The following are the characteristics of the fish used in the study: The average length of *Paralaubuca typus* was 11.08±3.33 centimetres; *Phalacronotus bleekeri* was 17.00±5.13 centimetres; and *Phalacronotus bleekeri* was 20.54±4.49 centimetres (Table 1).

Table 1: Species and number of fish samples caught in the Mun River

Species	Habitat	Food	Length (cm)	Number of samples
Paralaubuca typus	Surface	Insects, small aquatic animals.	11.08±3.33	24
Phalacronotus bleekeri	In the middle of the water	Insects, small aquatic animals.	17.00±5.13	24
Morulius chrysophekadion	Deep water	Plants, insects.	20.54±4.49	24

The average number and percentage of microplastic contamination in fish was 13.26 ±8.69 pieces/fish, microplastics found in *Morulius chrysophekadion* was 17.70±8.20 pieces/fish (45.33%), *Phalacronotus bleekeri* was 11.83±8.74 pieces/fish (27.51%), and *Paralaubuca typus* was 10.25±7.56 pieces/fish (27.16%), respectively (Figure 3). The average number and percentage of microplastic contamination in the urban area was 26.50±4.21 pieces/fish (49.95%); in the agricultural area was 9.77±4.10 pieces/ fish

(18.43%); in the confluence of the Mun and Chi River areas was 9.50±3.51 pieces/ fish (17.91%); and the natural area had 7.27±4.15 pieces/fish (13.72%), respectively. (Figure 3 & 4).

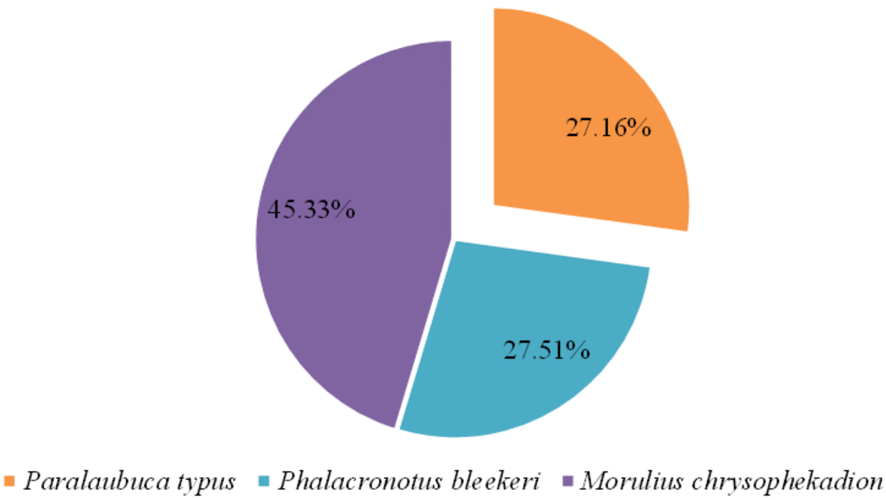


Figure 3: Percentage of microplastic contamination in fish by Species

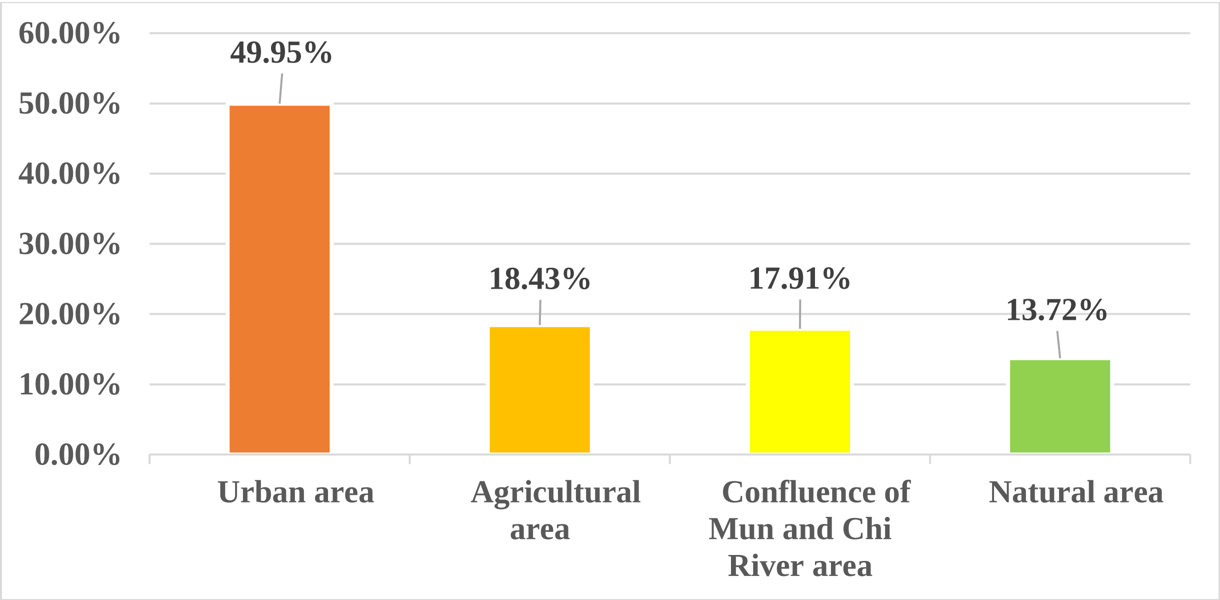


Figure 4: Percentage of microplastic contamination in fish by sampling sites

3.2 Size, shapes, and colors of microplastics

To identify the size, shapes, and colors of microplastics, a stereomicroscope was used. The size of microplastics ranged between 98-9423 µm. The average size of microplastics was 878.29±904.41 µm. *Morulius chrysophekadion* found the largest microplastic to be 9423 µm (Table 2). Microplastic sizes of 350 to < 500 µm were the predominant size in *Paralaubuca typus*; microplastic sizes of 500 to < 1000 µm were the predominant size in *Phalacronotus bleekeri* and *Morulius chrysophekadion* (Figure 5). The four shapes of microplastics in the contaminants of fish were identified as fiber, fragment, sheets/film, and rod (Figure 6 and Figure 7).

Table 2: Average size of microplastics

Species	Mean* ($\bar{X}$±S.D.)	Min	Max
Paralaubuca typus	862.01±739.62	153	5,784
Phalacronotus bleekeri	802.71±673.10	107	5,827
Morulius chrysophekadion	938.21±1101.96	98	9,423
Total	878.29±904.41	98	9,423

*The average size of microplastics (µm).

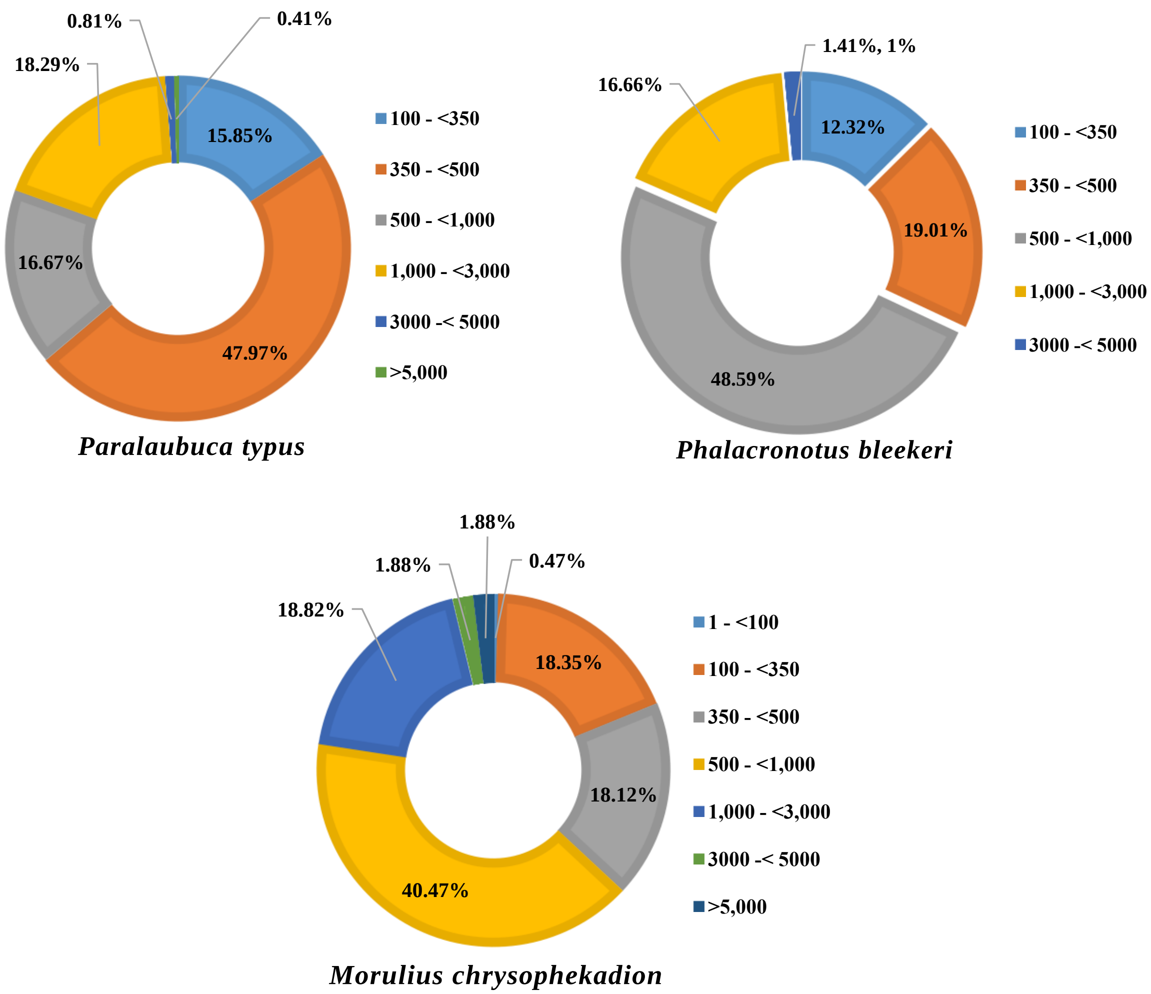


Figure 5: Percentage and size range of microplastic contaminants in each species

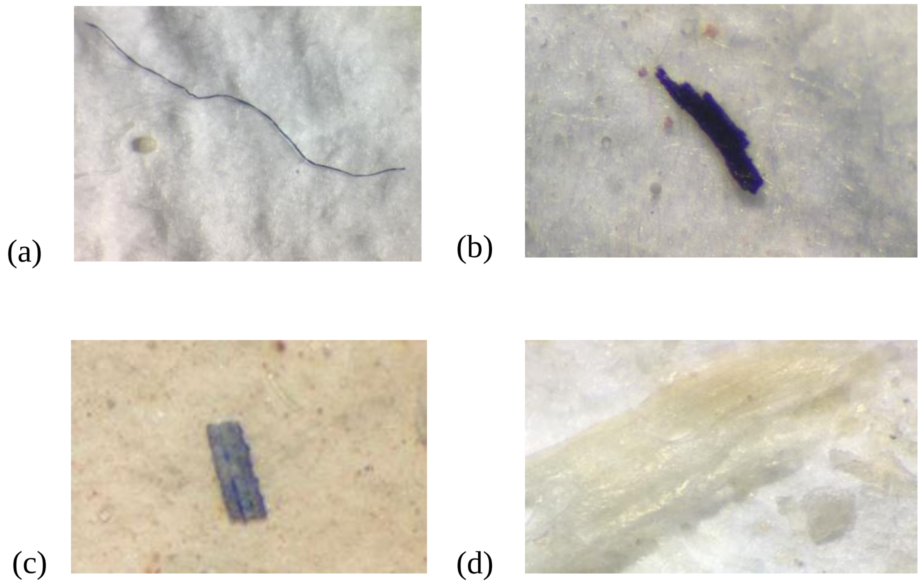


Figure 6: Shape of microplastics: (a) fiber, (b) fragment, (c) rod, (d) sheets/film

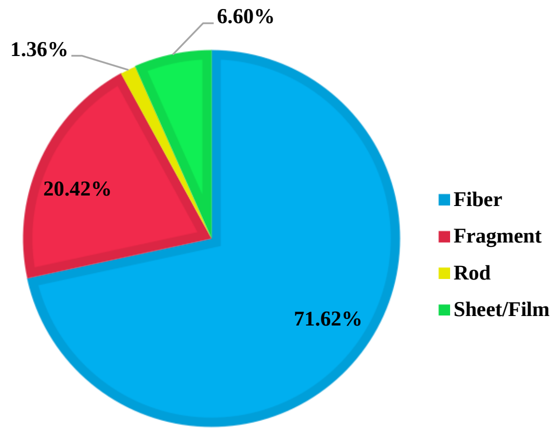


Figure 7: Percentage of microplastics by shape

Ten colours of microplastics were found in this study: blue (44.08%), purple (20.42%), pink (10.68%), black (8.27%), white (5.76%), transparent (5.67%), orange (3.25%), brown (1.26%), green (0.52%), and red (0.10%), respectively (Figure 8).

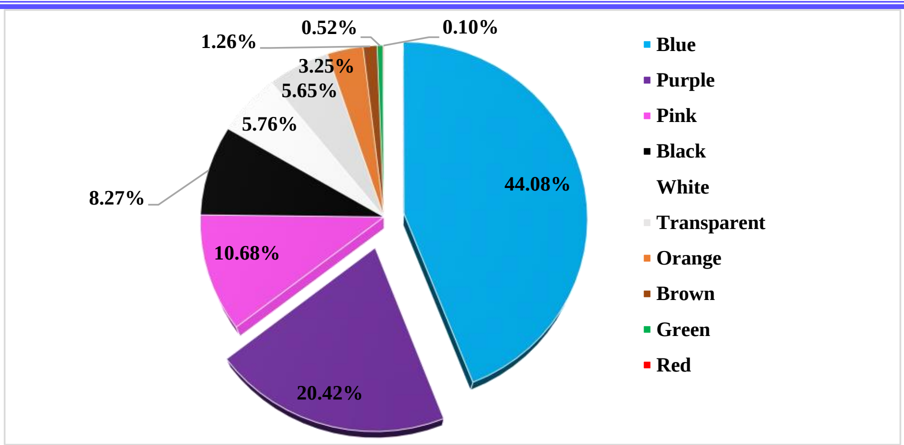


Figure 8: Percentage of microplastic by colors

3.3 Polymer types of microplastics

To identify the polymer types of microplastics found in the study, fourier transform infrared (FT-IR) spectroscopy was used. A total of seven polymer types of microplastics were contaminants: polyethylene (PE), followed by polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polystyrene (PS), polycarbonate (PC), and nylon, respectively (Figure 9).

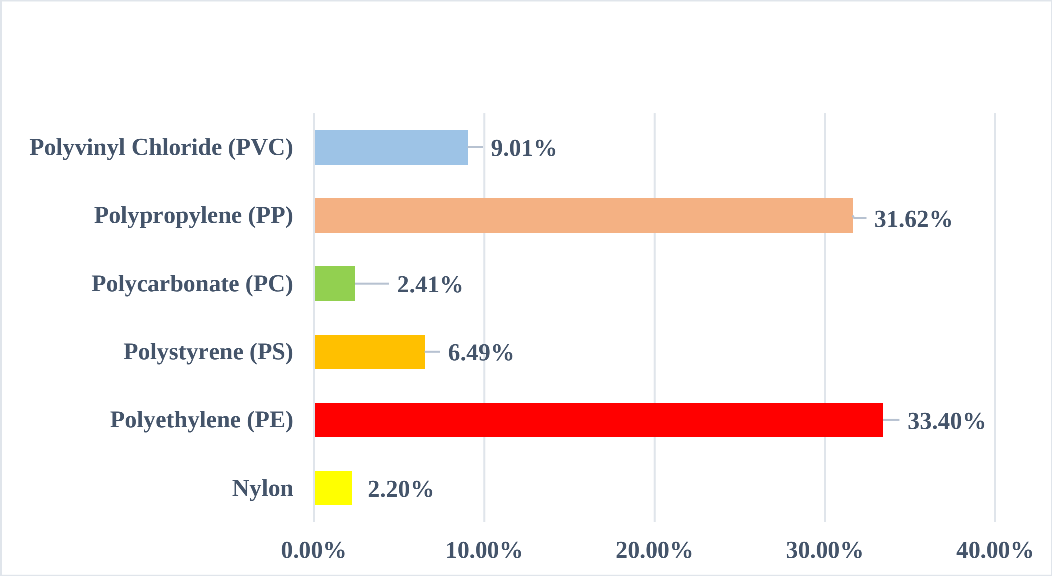


Figure 9: Percentage of polymer types of microplastics

4. Discussion

The average number of microplastics in fish were 7.87 ± 5.57 pieces/fish. The average number of microplastics was high when compared with fish from tropical estuaries of Brazil (0.12±0.37 particles/fish) [19], was higher than fish in the Pajeu River of Brazil

(3.6 particles/fish) [13], and higher than fish from the Chi River (1.76±0.58 particles/fish) [12], and the Nam Pong River (7.60 ± 17.70 particles/fish) [11], which is also in the Northeastern region. Most microplastic accumulation was seen in Morulius chrysophekadion. The findings were consistent with previous studies which found that the number of microplastics were correlated directly with fish length [20]. Larger fish are more likely to have the maximum ingestion because they consume more food than smaller fish, which is necessary to meet their energy needs [19]. This study also found that fish in urban areas have the highest contamination of microplastics. This is consistent with previous studies; microplastics in urban areas are high [21], [10], and consistent with the study of Yuan D et al. (2023), which found that microplastic abundance in urban stormwater inlet (USI) sediments was higher in commercial areas than agricultural [10]. In aquatic organisms, microplastics can have a variety of detrimental effects, including intestinal mucosa absorption and obstructions of the gastrointestinal tract. According to a study by Americans [22], microplastics pose an increasing threat to human health and food safety, in addition to aquatic animals. The presence of microplastic particles in the colon [23], stool [24] and even the bloodstream [25] is an accurate indicator of human ingestion of microplastics. The size distribution of microplastics ranged between 98-9423µm. The size of the particles has a significant influence on the level of biological toxicity of microplastics, smaller microplastic particles can be internalized by organisms by endocytosis by honeycomb cells, whereas larger particles can be taken up by macrophages in the small intestinal epithelium through phagocytosis [13]. According to Lusher H and Hill M (2017) microplastic (0.1–150 µm; human: 0.2–150 µm) can be transported to the lymphatic system of mammals. Fiber was the most prevalent form of microplastic accumulation in fish. The shape results concurred with former studies conducted in Taihu Lake of China [18], Chi River of Thailand [12] and Bueng Borapat Wetland of Thailand [10] in Bursa. An important source of fiber microplastics is wastewater [26]. The most common colour contamination is blue in fish. The abundant color results concurred with former studies in Nam Pong River [11], Chi River [12] and at freshwater lakes (Taihu Lake) in China [18]. Because of a visual misunderstanding between food, fish might consume microplastics [27]. The most common type of microplastic polymer accumulated in the study was polyethylene (PE) which is consistent with studies of microplastics in indigenous freshwater fish in the south of Italy [28]. Polyethylene fiber indicates that it is the primary source of microplastics in wastewater [29]. The characteristics and polymer type of microplastics in the fish ingested remained consistent across wet and dry seasons [30].

5. Conclusion

This study found significantly higher microplastic contamination in fish compared to various other studies, with *Morulius chrysophekadion* showing the highest accumulation. Larger fish and those from urban areas were more impacted. The dominant type was fibrous polyethylene, potentially originating from wastewater. Microplastics pose potential threats to human health due to ingestion and detrimental effects on aquatic organisms.

In light of the potential health risks posed by microplastics, agencies responsible for public health should provide knowledge about microplastics and offer recommendations for reducing exposure to microplastics, including from fish consumption. Removing the intestines and gills of fish before consumption can reduce exposure to microplastics.

Acknowledgement

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