ORIGINAL ARTICLE

Microplastic contamination in fresh fish: insights from wet market in Selangor, Malaysia

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Received: 2 July 2024 **Revised:** 25 November 2024 **Accepted:** 26 November 2024 **Available online:** May 2025
DOI: 10.55131/jphd/2025/230215

ABSTRACT

The accumulation of microplastics (MPs) in fish has emerged as a significant concern, as it poses potential risks to aquatic ecosystems and human health. This study aimed to determine the presence of MPs in the gut and muscle tissue of fresh fish from Pasir Penambang wet market, Kuala Selangor, Malaysia. In this study, microplastic polymers from *Lutjanus erythropterus, Atule mate, Pampus argenteus* and *Rastrelliger kanagurta* were quantified and analyzed by Fourier transform infrared spectroscopy – attenuated total reflectance (ATR-FTIR). The highest MPs abundance (in both gut and muscle) was found in *L. erythropterus* (8.23 ± 11.57 P/gram of tissue), followed by *A. mate* (4.80 ± 3.61 P/gram of tissue), *R. kanagurta* (3.40 ± 3.61 P/gram of tissue), and *P. argenteus* (2.70 ± 1.85 P/gram of tissue). Fiber was the most dominant shape in both the gut (73.2%) and muscle tissue (81.4%). Black was the most abundant color in both the gut (51.79%) and muscle tissue (54.64%). High amounts of MPs were found with sizes smaller than 0.5 mm in the gut (57.44%) and muscle (42.27%). Polyethylene terephthalate (PET) is the most abundant polymer, with polypropylene (PP) and cellulose acetate following closely behind. Additionally, an estimated annual intake (EAI) of around $5.445x10^{-3}$ to $3.8445x10^{-2}$ P/kg/bw/year and $9.075x10^{-4}$ to 6. $4075x10^{-3}$ P/kg/bw/year were determined for two groups of adults and children, respectively. MPs can accumulate in the gastrointestinal system of fish upon ingestion and subsequently migrate to various tissues inside the body. This study highlights the ubiquitous extent of MPs pollution in marine ecosystems and assesses its potential consumption through the human diet.

Keywords:
microplastics; fish; gut; muscle tissue; wet market

Citation:
Nor Azlan AS, Yatim SRM, Osman MS, Rasdi NW, Shafie FA, Porusia M. Microplastic Contamination in fresh fish: insights from wet market in Selangor, Malaysia. J Public Hlth Dev. 2025;23(2):207-221 (https://doi.org/10.55131/jphd/2025/230215)

INTRODUCTION

The global production of plastic currently stands at an average of 430 million tons annually.[1] Plastic is light, moldable, robust, and inexpensive as well as a perfect material for many purposes. However, the effects of today's single-use plastic culture are obvious on coastlines and in oceans worldwide. Once the plastic enters the oceans, it will last out for a very long time, and it just breaks down into smaller and smaller pieces which are known as microplastics (MPs). MPs are plastic particles that are less than five millimeters in length and can be a threat to our environment and the aquatic ecosystem.

MPs can be divided into two groups: primary and secondary microplastics. Primary MPs are from industrial sources produced in microscopic proportions including cosmetics products[2], industrial materials[3], and cleaning products[4]. Secondary MPs are the outcome of physical, chemical, and biological degradation of larger plastic debris caused by light degradation, UV radiation, embrittlement, and biological degradation.[5] Today, MPs are invading much more of the ocean than aquatic life. Due to their small size and vibrant colors, many marine species mistakenly consume them, assuming them as their natural prey.[6] Studies have consistently indicated that MPs can induce a range of adverse health effects in fish, which include immune response, oxidative stress, neurotoxicity, and reproductive problems.[7]

According to the World Health Organization (2011), fish is one of the basic protein diets for humans, and officials have long urged individuals to consume more fish.[8] MPs are thought to be consumed by fish because apparently fish mistake microplastics for prey and then consume them. This phenomenon is considered to be very common. MPs have the potential to contaminate either fresh fish[9] or canned fish[10]. Additionally, MPs constitute a threat to marine species and the natural habitats in which they live[11], and this may have additional implications for the safety and security of food supplies.[12]

As fish are a vital source of protein for many Malaysians, MPs in seafood present direct risks to human health, including potential toxicological effects. With MPs known to accumulate in fish tissues, there is an urgent need to quantify their presence and assess human exposure risks, especially in areas heavily reliant on local fisheries like Kuala Selangor. This research introduces a focused assessment of microplastic (MP) contamination in fish species from the Pasir Penambang wet market in Kuala Selangor, Malaysia, which has not been extensively studied. By evaluating both the abundance and characteristics of MPs in fish gut and muscle tissue and estimating human exposure through the estimated annual intake (EAI) formula, the study adds critical insights into the pathways of MPs entering the food chain. Furthermore, it contextualizes local findings with broader environmental and health implications, serving as a bridge between localized contamination and global concerns.

Understanding the extent of MPs contamination in fish within this area is crucial for assessing the environmental and human health impacts of MPs. Therefore, this study aims to assess the abundance and characteristics of MPs in the gut and muscle tissue of selected fish species. The outcome of this research will be especially beneficial for the government, especially for the National Solid Waste Management Department (NSWMD) in proposing policies, plans, and strategies to successfully deal with the MPs problem since plastic waste is managed by this department. In addition, Malaysia has implemented several microplastic policies and initiatives, including the Roadmap Towards Zero Single-Use Plastics 2018–2030 [13] and the National Marine Litter Policy and Action Plan (NMLPAP) 2021–

2030.[14] In summary, this research aligns with urgent global and national needs to address plastic pollution and its cascading effects on ecosystems, human health, and policy planning. It is a step forward in filling knowledge gaps and supporting Malaysia's journey toward sustainable plastic waste management.

MATERIALS AND METHODS

Sample collection

Four species of fish (*Rastrelliger kanagurta*, *Atule mate*, *Lutjanus erythropterus* and *Pampus argenteus*) (Figure 1) were collected from the sampling location in Pasir Penambang wet market, Kuala Selangor, Malaysia. These particular fish species were among the most popular among consumers. All the collected samples have nearly the same weight (g) and body length (cm). The samples collected were immediately wrapped in aluminium foil and transported to the laboratory in an icebox. Samples were immediately stored in the freezer at -20°C to ensure good preservation conditions before conducting further analysis.

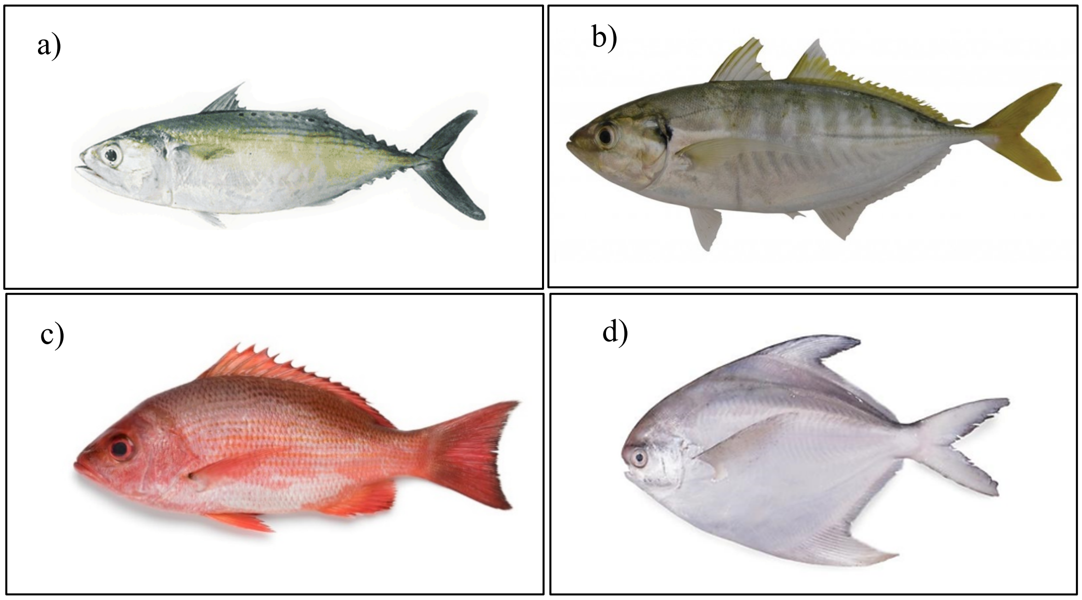


Figure 1. The selected fish species: a) *Rastrelliger kanagurta*, b) *Atule mate*, c*) Lutjanus erythropterus,* and d) *Pampus argenteus*

The first step involves the thawing of the fish samples. A cleaned metal tray was used to thaw and prepare the fish for dissection. Basic measurements were taken including the weight, measured in grams (g), and the body length, measured from the mouth to the central point of the caudal fin, expressed in millimeters (mm). Using a clean scalpel and scissors, gut and muscle sub-samples were minced. The gut and sub-samples of muscle from the mid lateral section of each individual were removed after making a longitudinal opening in the abdominal area. After that, 5 g of minced gut and muscle tissues were prepared for digestion. The applied method of digestion and MPs extraction procedures were carried out based on Li et al. (2018).[15]

The separated gut and muscle were transferred to a 1 L conical flask labeled with the species name and organ name. 200 mL of 30% hydrogen peroxide (H_2O_2) was added to the conical flask which was then securely covered with aluminum foil to digest the samples. The conical flask was incubated at 65°C for 24 hours. It was gently shaken several times to enhance the digestion process. After incubation, the samples were then allowed to rest at room temperature for an additional 24 to 48 hours. Once a clear appearance was achieved, 800 ml of sodium chloride (NaCl) solution was poured into each conical flask and then it was left to settle for a day. Using a vacuum system, the surface water was carefully removed and then filtered through a cellulose nitrate membrane filter with a pore size of 0.45 µm and a diameter of 47 mm. Finally, the membrane filter with MPs was carefully transferred to a covered petri dish and allowed to dry until further analysis.

Microplastics identification

Microplastics (MPs) characteristics were observed and recorded based on their shapes, sizes, and colors. The residues on filter paper were visually examined using the ZEISS Stemi 305 stereomicroscope to identify the shapes and colors of MPs. The sizes were analyzed and measured using a Dino-Lite Edge AM7915 Series and Dino Capture 2.0 software. MPs were classified into four different categories consisting of fibers, fragments, films, and pellets; with three size groups which are less than 0.5 mm, 0.5–1.0mm, and 1.0–5.0mm. Furthermore, the samples were submitted for analysis using Fourier transform infrared spectroscopy – attenuated total reflectance (ATR-FTIR) in order to verify the specific types of microplastic polymers present. The MPs were spread on a crystal of potassium bromide (KBr). The observations were taken in transmission mode at wavenumbers ranging from 400 to 4000 cm^{-1}.

Quality control

To avoid sample contamination, all procedures are followed consistently throughout the experiment. All laboratory equipment was thoroughly washed with distilled water and securely packed in aluminum foil until needed. To eliminate plastic contamination, all sample and analysis processes were carried out using glassware, metal spoons, and metal trays.

Data analysis

SPSS software (version 27.0) was used to conduct the statistical analysis. Normality test was used to determine the suitable tests that can be applied based on the collected data. Since the results indicated that the data failed the normality test, the Kruskal-Wallis (KW) test was used to compare the abundance differences of MPs among species while the Mann-Whitney U test was used to analyze the variation in MPs between the gut and muscle tissues of each species.

Human exposure

To determine the degree of human exposure to MPs, the annual intake index was calculated. The calculations for this study were all based on Makhdoumi et al. (2021).[16] The mean amount of MPs per kilogram of fish muscle per year, which is also known as the estimated annual intake (EAI) was used to figure out how much MPs were exposed to humans. This equation may not be attributed to a single individual or source, but it has been utilized and referenced in numerous scientific studies, research articles, and environmental assessments focused on MPs pollution and its impacts on ecosystems. [10-12] The EAI is calculated using the formula:

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

where IR is the amount of fish muscle eaten each year per person, and C is the number of MPs per kilogram (Particle items/kg) or the weight of microplastics per kilogram (mg/kg). IR was the recommended meal size, with 16.5kg and 2.75kg fish muscle/year for adults and children, respectively.[16]

RESULTS

Microplastics abundance in the gut and muscle tissue

All the analyzed fishes contained microplastics (MPs), with the gut showing a significantly higher concentration compared to muscle tissue. Table 1 shows the abundance of MPs (mean ± SD) in the gut and muscle tissue. The highest MPs abundance was found in *Lutjanus erythropterus* (8.23 ± 11.57 P/gram of tissue), followed by *Atule mate* (4.80 ± 3.61 P/gram of tissue), *Rastrelliger kanagurta* (3.40 ± 3.61 P/gram of tissue), and *Pampus argenteus* (2.70 ± 1.85 P/gram of tissue). The fish with the most microplastic particles found in both the gut and muscle tissues was *L. erythropterus*. However, the least MPs were found in the gut of *P. argenteus* (3.67 ± 1.86 P/gram of tissue), while in the muscle tissue, they were found in *R. kanagurta* (0.33 ± 0.31 P/gram of tissue).

Table 2 indicates there was no statistically significant difference between the numbers of MPs in the gut and muscle tissues of all species (Mann-Whitney U test; p > 0.05).

Table 1. Abundance of microplastic particles (mean ± SD) in the gut and muscle tissue (MPs (Particles)/g of fish tissue) of the fresh fish samples

Samples	**MPs Abundance (MP (items)/g of fish tissue)**	
	Specific Tissue	**Mean ± SD**
Rastrelliger kanagurta	Muscle	0.33 ± 0.31
	Gut	6.47 ± 2.05
	Total	3.40 ± 3.61
Atule mate	Muscle	2.07 ± 1.94
	Gut	7.53 ± 2.53
	Total	4.80 ± 3.61
Lutjanus erythropterus	Muscle	2.33 ± 1.03
	Gut	14.13 ± 15.15
	Total	8.23 ± 11.57
Pampus argenteus	Muscle	1.73 ± 1.51
	Gut	3.67 ± 1.86
	Total	2.70 ± 1.85

Table 2. Mann-Whitney U test

Fish species	**Mann-Whitney U**
Rastrelliger kanagurta	(0.05, P<0.05), no significant differences
Atule mate	(0.077, P<0.05), no significant differences
Lutjanus erythropterus	(0.05, P<0.05), no significant differences
Pampus argenteus	(0.261, P<0.05, no significant differences

The shape, color, and size of microplastics

Microscopic images of the relevant shapes of MPs are shown in Figure 2. Percent portion of MPs in the gut and muscle tissues of fresh fish is shown in Figure 3. Four shapes were observed in the gut: fiber, fragment, pellet, and film; while only fiber and fragment were identified in the muscle tissue. Fiber was the most dominant shape found in both the gut

(73.2%) and muscle tissue (81.4%) of the fish species, followed by fragment form, with the gut containing 20.3% and the muscle tissue containing 18.6%. Pellet (5.7%) and film (0.8%) were only found in the gut, while none in the muscle.

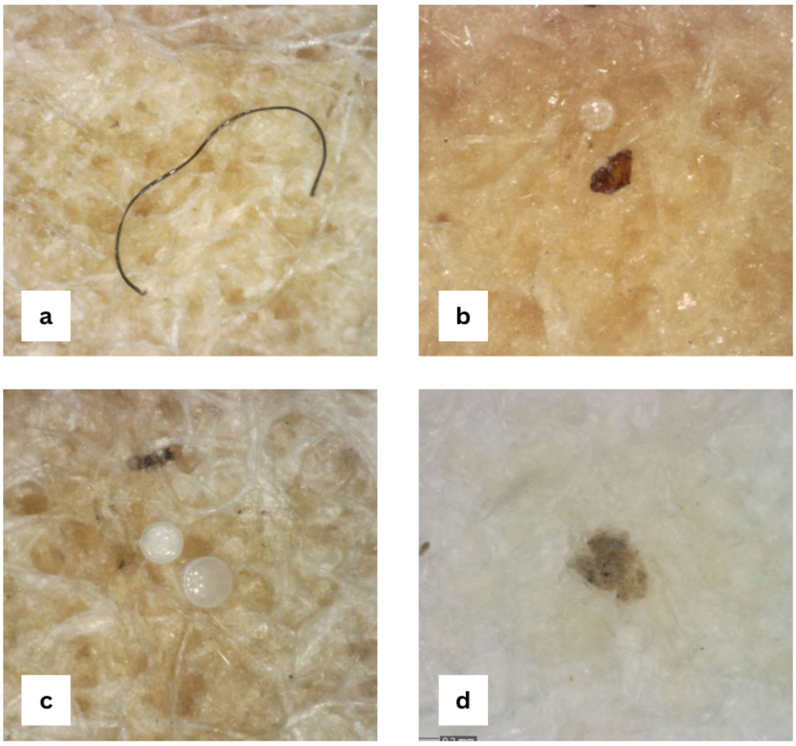
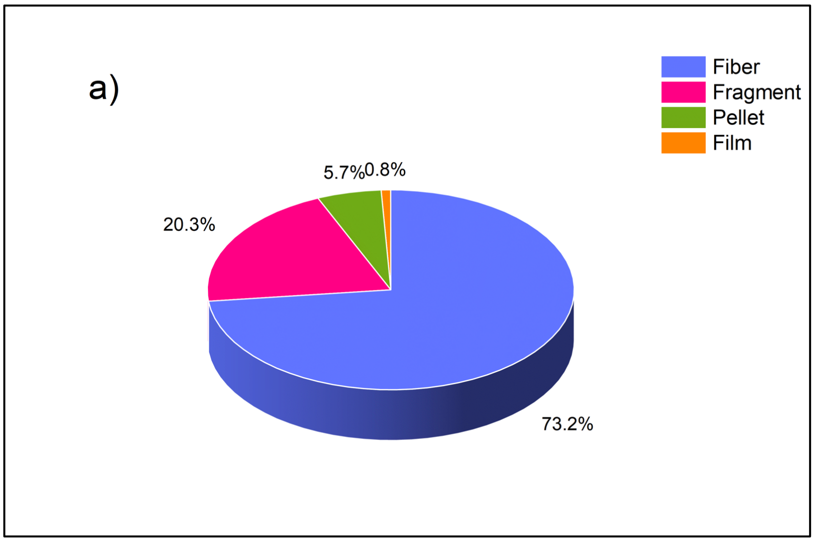


Figure 2. Microscopic images of selected MPs: a) fiber, b) fragment, c) pellet, and d) film



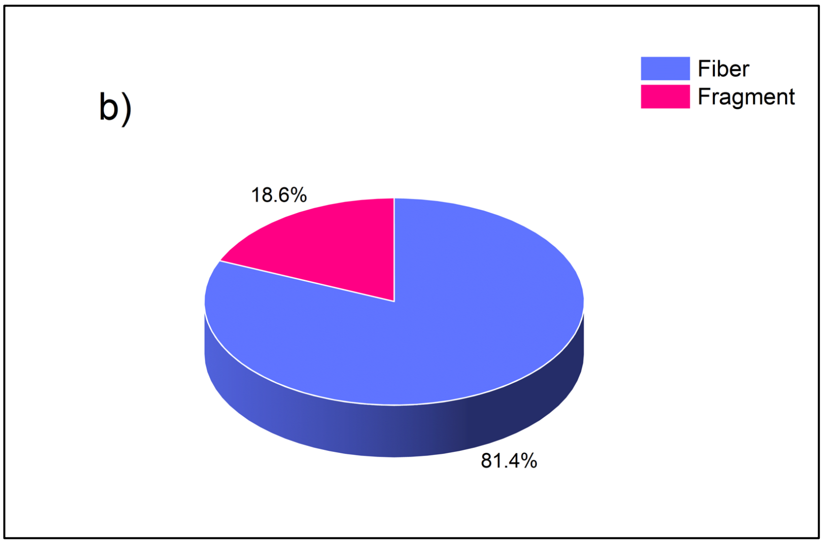


Figure 3. Shapes of MPs in the gut (a) and muscle tissue (b)

This research discovered nine colors of MPs in this study: black, purple, green, yellow, red, transparent, blue, brown, and also colorful. As shown in Figure 4**,** black color was the most abundant in both the gut (51.79%) and muscle tissue (54.64%). The remaining colors in the gut were red (15.3%), green (7.34%), brown (6.29%), white (5.66%), blue (4.4%), yellow (3.98%), transparent (3.77%) and purple (1.47%). As for the muscle tissue, it was followed by blue (12.37%), red (9.28%), yellow (6.19%), brown (5.15%), purple (4.12%), transparent (4.12%) and green (4.12%).

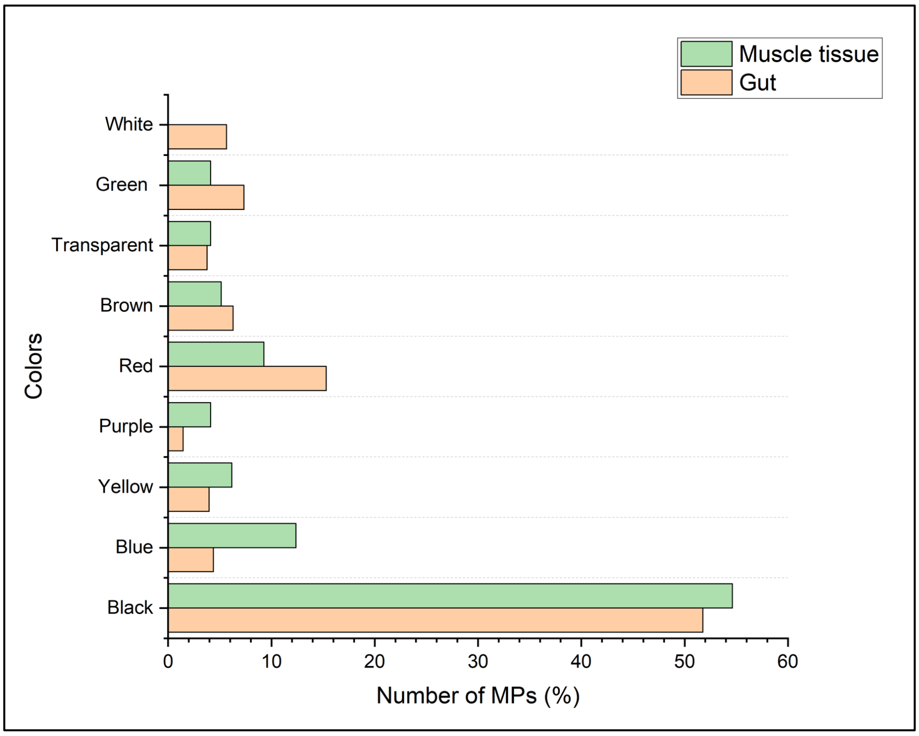


Figure 4. Colors of MPs in the gut and muscle tissue

Size categories of extracted MPs from the gut and muscle tissues of fish species are shown in Figure 5. MPs of less than 0.5 mm were the most abundant in the gut and muscle tissues, with a percentage of 57.44% and 42.27%, respectively. MPs found in the gut with size ranges between 0.5 to 1.0 mm (24.95%) were the second highest while those in the range between 1.0 to 5.0 mm (17.61%) were the least.

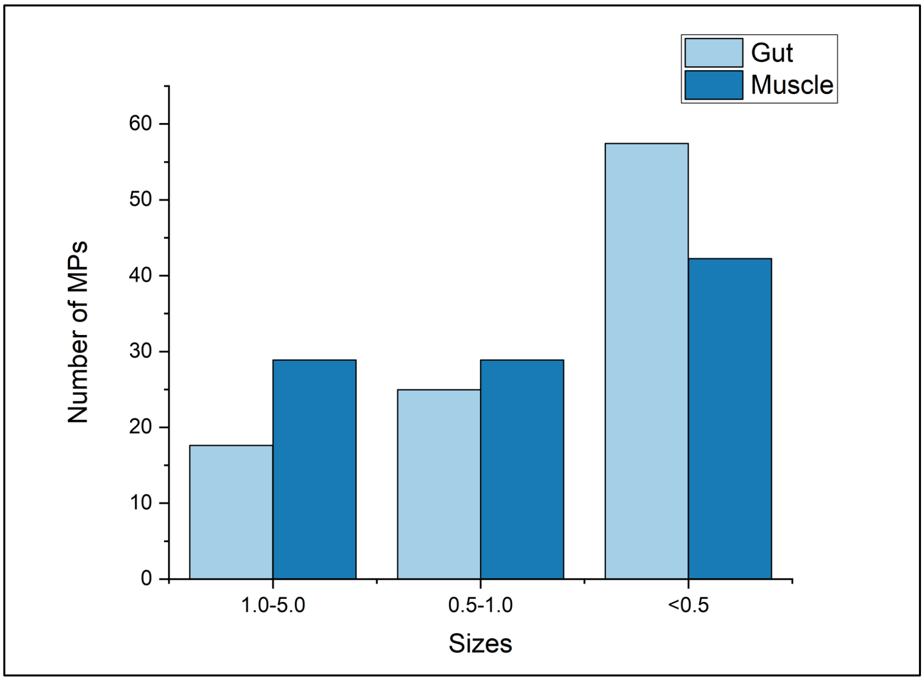
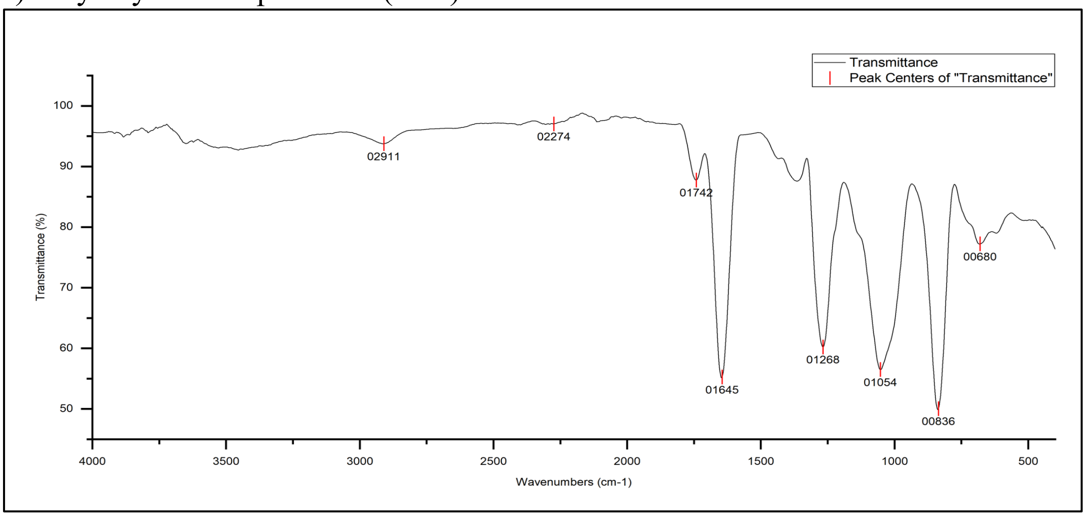


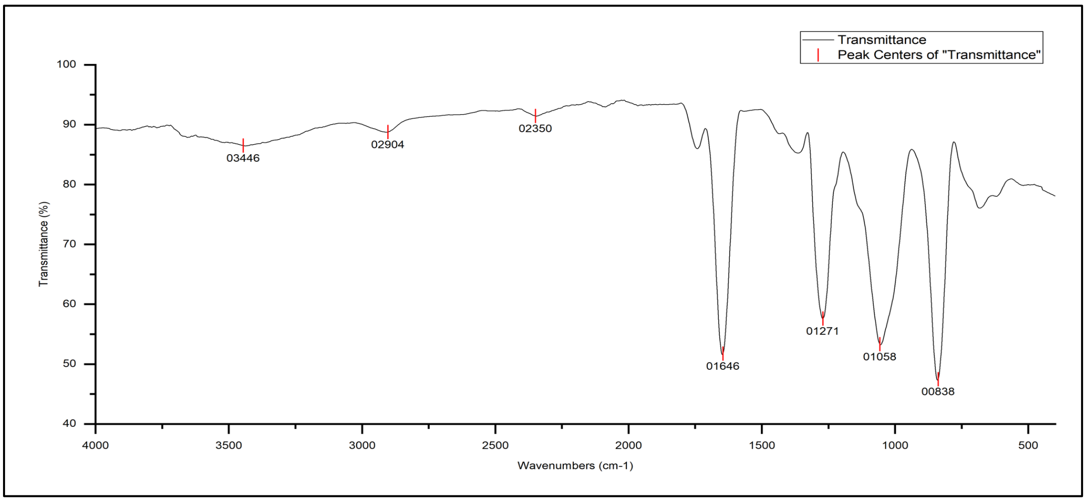
Figure 5. Sizes of MPs in the gut and muscle tissue

Polymer composition of MPs

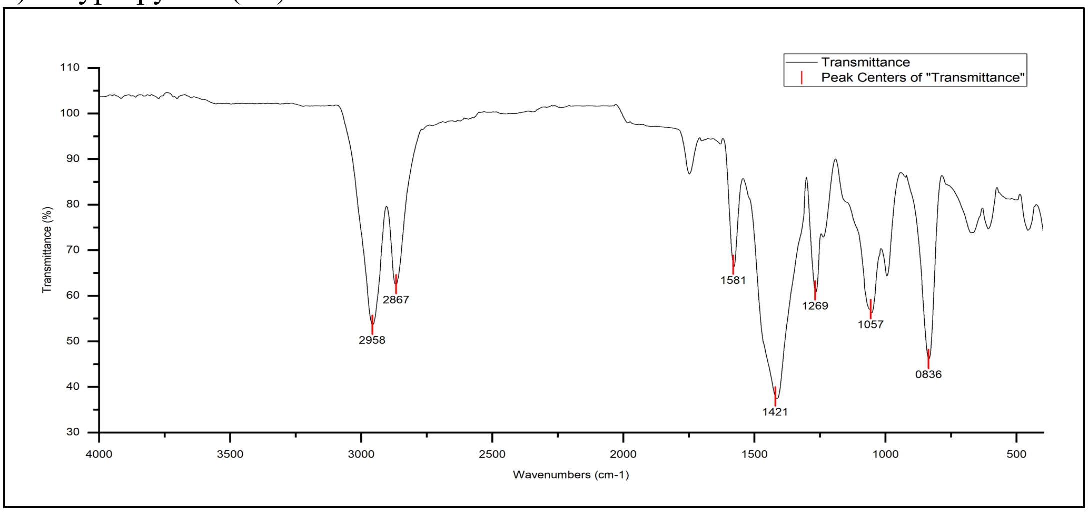
ATR-FTIR analysis was used to identify the main polymer types of detected MPs in the muscle tissue of each species with a total of eight samples. The polymers identified were polyethylene terephthalate (PET), polypropylene (PP), and cellulose acetate. Figure 6 shows PET as the most dominant polymer found in the muscle tissue, followed by PP and cellulose acetate.

a) Polyethylene terephthalate (PET)





b) Polypropylene (PP)



c) Cellulose acetate

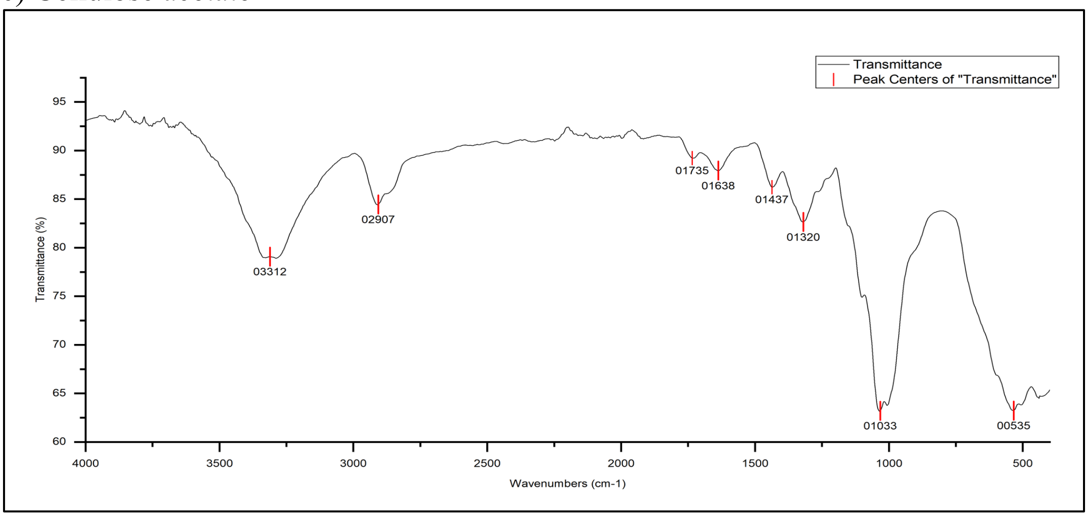


Figure 6. ATR-FTIR spectra of the MPs found in the representative samples: a) PET, b) PP, and c) cellulose acetate

Human exposure

The EAI (MP/kg consumed muscle of fish/year) result for adults and children is displayed in Table 3. According to the following outcome, an intake of around $5.445x10^{-3}$ to $3.8445x10^{-2}$ P/kg/bw/year and $9.075x10^{-4}$ to 6. $4075x10^{-3}$ P/kg/bw/year were estimated by two groups of adult and children, respectively. The EAI of *Lutjanus erythropterus* for adults and children is the highest compared to all other species, while *Rastrelliger kanagurta* has the lowest recorded EAI. The consumption rate of MPs after taking *Lutjanus erythropterus* was $3.8445x10^{-2}$ P/kg/bw/year for adults and 6. $4075x10^{-3}$ P/kg/bw/year for children, while in the case of *Rastrelliger kanagurta*, the intakes were $5.445x10^{-3}$ P/kg/bw/year for adults, and $9.075x10^{-4}$ P/kg/bw/year for children.

Table 3. EAI for adults and children

Species	MPs Concentration (particles/kg)	Group/Body Weight (kg)	EAI (P/kg/bw/year)
Rastrelliger kanagurta	$3.3x10^{-4}$	Adults	$5.445x10^{-3}$
		Children	$9.075x10^{-4}$
Atule mate	$2.07x10^{-3}$	Adults	$3.4155x10^{-2}$
		Children	$5.6925x10^{-3}$
Lutjanus erythropterus	$2.33x10^{-3}$	Adults	$3.8445x10^{-2}$
		Children	6. $4075x10^{-3}$
Pampus argenteus	$1.73x10^{-3}$	Adults	$2.8545x10^{-2}$
		Children	$4.7575x10^{-3}$

DISCUSSION

The present study confirms the occurrence of microplastics (MPs) in both the gut and muscle tissue of the fresh fishes collected from the Pasir Penambang wet market in Kuala Selangor, Malaysia. Although there may not be much of a difference, the amount of MPs in the gut was shown to be greater than in the muscle tissues, which aligns with earlier studies.[17] Fish can unintentionally ingest and accumulate MPs through their digestive systems.[18] Given that the gut is a component of the digestive system, the higher concentration of MPs in the gut can be rationalized.

Based on Table 3, there was only a slight variation in the distribution of MPs between species, potentially due to the habitat and feeding habits of the fish. *Rastrelliger kanagurta* and *Atule mate* are classified as pelagic fish (open water fish), while *Lutjanus erythropterus* and *Pampus argenteus* are classified as demersal fish (sea bottom fish). This study has revealed that demersal species had a higher concentration of MPs compared to pelagic fish. MPs tend to sink and accumulate in sediments, where demersal fish feed, making them more exposed to ingestion through food or direct sediment intake. However, it is worth noting that other studies have shown that pelagic fish consumed more MPs compared to demersal fish.[13] Pelagic fish, which eat plankton, may easily devour MPs since they are small, lightweight, and float in the ocean.[19] *L. erythropterus* exhibited a higher concentration of MPs compared to the other pelagic species; it may be attributed to the proximity of plastic debris to the seafloor in the species' habitat. Regardless, it should be pointed out that comparing research is challenging due to variations in the techniques applied to separate and quantify MPs, as well as the amount of tissue analyzed.[20]

The abundance of fiber in this study indicates the considerable influence of fibrous MPs on the marine environment. Other studies have also found that fibers are the most common shape of MPs in fish.[21] The primary sources of MPs found could be attributed to effluents from fishing gear, such as nets and ropes, along with discharge from washing machines. About 18% of ocean plastic comes from abandoned, lost, or discarded fishing gear. [22] Moreover, a single piece of clothing can generate over 1900 fibers in a single wash, and the total amount of fibers released into wastewater may exceed 100 fibers per liter.[23] Therefore, the fragmentation of these materials can result in the release of fibers into the ocean, which can be unintentionally ingested by fish.

The prevalence of black-colored MPs is consistent with prior research.[21] The widespread use of black-colored plastic goods, primarily in food packaging, cooking utensils and appliances, toys, electrical home appliances, and automobile components might be the main reasons.[24] Certain plastics may possess colors that resemble the visual characteristics of small organisms or particles that are commonly ingested by fish. Therefore, colored plastic particles, especially those that reflect the color and size of natural prey, have the ability to visually attract fish and other marine organisms.[21]

Besides that, this study found that MPs less than 0.5 mm were the most abundant, which is consistent with other previous studies.[25] While the exact sizes of MPs in fish that can pass through the human body and cause harm are not yet known, the past literature has proved that MPs of various sizes can enter the human body without even realizing it, especially those measuring smaller than 0.5 mm.[26-27]. MPs contain substances that act as endocrine disruptors, potentially leading to significant adverse effects on the well-being of individuals.[27] Thus, when contaminated food and water are consumed, the substances might be absorbed into the human body and subsequently disrupt the human body such as the endocrine, reproductive, and digestive systems.

In this study, polyethylene terephthalate (PET) was the most common polymer detected in the muscle tissue of the representative samples, followed by polypropylene (PP) and cellulose acetate. PET is widely utilized in the production of a variety of goods, such as plastic bottles, packaging bags, drinking straws, milk jugs, and textiles.[28] Potential sources of PP are rather comparable to PET, possibly due to its widespread utilization in food and beverage packaging, plastic containers, and wrapping plastics. PP may also originate from the wear and tear of fishing instruments, such as fishing nets, rods, reels, and other fishing equipment, as they are extensively used in global fishery activities due to their durability.[29] Therefore, the combination of the widespread use and slow degradation of PET and PP make them relatively more abundant in the marine environment compared to cellulose acetate. Although cellulose itself is not categorized as a plastic, cellulose acetate can be considered as bioplastics or biodegradable polymers typically used in cigarette filters.[30] The filters contain elements of harmful substances such as nicotine, cadmium, and various other chemicals which have the potential to contaminate the environment when they are disposed of.[31] From this study, it can be observed that only PET, PP, and cellulose acetate were present, compared to other studies. This can be attributed to differences in location and sources of pollution, which vary from those in other studies. As stated in a previous study, the types of plastics identified in samples may be influenced by their geographical location and environmental conditions in which different forms of plastic are used or disposed of.[32]

The Daily Intake (EDI) was calculated to measure human exposure to microplastics from fish intake. The figure only shows the exposure of MPs and the influence of embedding compounds connected to plastic particles, neglecting conductive additives. However, these additions might cause health problems such as cytotoxicity, inflammation, and accumulation in lipophilic tissues, as well as a slow clearance rate from the body. It is critical to realize the various recommended levels for microplastic consumption, as well as the lack of exact evidence on the toxicity of specific plastic types in the human body. As a result, the EAI assessed in this study cannot fully capture the significance of exposure risks to human health in this country.

Microplastics in seafood cause significant health risks, as they can be swallowed by marine species and then digested by humans. Consumption of contaminated seafood by humans may result in the ingestion of microplastics, which may contain detrimental substances such as persistent organic pollutants (POPs) and endocrine-disrupting chemicals (EDCs).[34] These chemicals may present health hazards, including hormone imbalances, reproductive complications, immune system disturbances, and maybe cancer[35,36]. The prolonged consequences of microplastic exposure via seafood consumption remain under investigation.

CONCLUSION

The findings from this research validate that MPs have penetrated the internal organs of marine fish. MPs were predominantly detected in the gut and muscle tissue of examined fish species: *Rastrelliger kanagurta, Lutjanus erythropterus*, *Atule mate,* and *Pampus argenteus*. The common shape, color, and size of the MPs were fibers, black, and smaller than 0.5 mm, respectively. The ATR-FTIR analysis revealed the polymers of the representative samples as PET, PP and cellulose acetate. This study highlights the ubiquitous extent of MPs pollution in the marine environments and assesses the potential consumption in the human diet. As this study solely addressed the annual consumption of MPs by individuals without adequately investigating the potential risks it may pose to human health, it is vital to conduct more studies on the prevalence and potential risks of MPs in muscle tissue, as it is commonly ingested portion of fish by humans.

ACKNOWLEDGEMENT

The authors would like to thank the laboratory staff of the Centre for Environmental Health and Safety Studies, FSK UiTM for their assistance.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ETHICAL CLEARANCE

The ethics were approved by the Research Ethics Committee with reference number FERC/FSK/EM/2023/00037.

FINANCIAL SUPPORT

No funding was received for this work.

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