Microplastic contamination in commercial marine fish: A case study in Johor, Malaysia

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Abstract. Microplastic contamination in marine ecosystems endangered marine organisms such as fish and poses a risk to humans. This research aims to investigate the presence of microplastic contamination in commercial marine fish caught around Johor, Malaysia. This study uses samples from four species of commercial marine fish consists of Indian mackerel, Yellowtail scad, Forktail threadfin bream and Black pomfret. Furthermore, microplastics were extracted, characterized, and identified from fish flesh. The results show that the fish species with the highest number of microplastics were yellowtail scad (23.33%) and Indian mackerel (30%) from all fish analyzed, which had an average of 0.022 and 0.021 particles/g, respectively. The pelagic fish has a higher microplastic number than the demersal fish (p-value = 0.037). Black fragments with < 200 µm in size are the majority of microplastics discovered. Fish flesh predominantly contains microplastics like polyamide (PA) and Ethylene propylene diene monomer (EPDM). Further study and regular monitoring on microplastic contamination in commercial marine fish need to be done to mitigate the impact of microplastics on human health and marine ecosystems, particularly in Johor, peninsular Malaysia.

1 Introduction

Microplastic pollution has emerged as a serious environmental concern across the world, particularly in marine areas where it endangers marine life and human health. Microplastics, which are plastic particles smaller than 5 mm, originate from many sources such as the fragmentation of bigger plastic waste, synthetic fabrics, and personal care items [1]. Because of their microscopic dimensions, microplastics can be consumed easily by marine organisms, including commercial marine fish species, therefore possibly entering the human food chain [2].

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Johor is one of the states in peninsular Malaysia that is situated close to the South China Sea, which serves as a major maritime route and locations several fisheries activities, including fish landing ports along the coast such as the Malaysian Fisheries Development Authority (LKIM) Endau. Moreover, this state was the second-highest producer of fisheries on the east coast of peninsular Malaysia and the seventh-highest producer in Malaysia in 2023, with an estimated 77,773 metric tonnes [3]. Furthermore, there are estuaries formed by major rivers along the coast, including the Endau River, Mersing River, and other smaller rivers that may carry materials from urban areas along the river, including plastic. In addition, there are also many tourist attractions, such as Tinggi island and Sibu island [4]. All of these factors may contribute to the microplastic contamination in the area. The existence of microplastics in marine fish in coastal areas, such as Johor, Malaysia, where fishing plays a vital role in the economy, gives rise to concerns over the safety and long-term viability of seafood. Therefore, quantifying the level of microplastic contamination in commercial marine fish species is crucial for evaluating the possible hazards to human health and for formulating measures to mitigate this environmental issue.

The objective of this research is to examine the number, shapes, sizes, types, and colours of microplastics present in several commercial marine fish species collected from the marine waters around Johor, Malaysia. This study intends to add to the greater knowledge of marine pollution in peninsular Malaysia and give insights into the possible health concerns connected with seafood intake by analysing the microplastic contamination in these fish.

2 Methods

2.1 Sample collection

The samples were collected from the Malaysian Fisheries Development Authority (LKIM) Endau and Pasar Tani Kekal (PTK) Endau, Johor in July 2023. Moreover, LKIM Endau is one of the main fish landing ports in Peninsular Malaysia (Fig. 1).

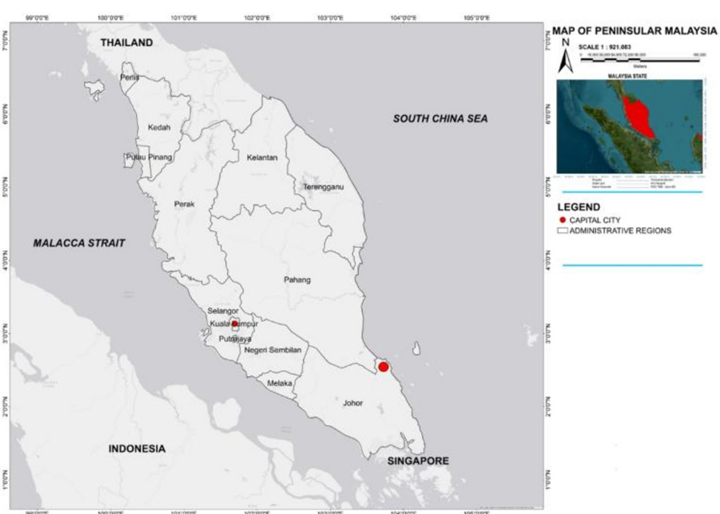

Fig. 1. Map of sampling location.

This study used a total of 120 fish consisting of two pelagic fish species ((Indian mackerel (*Rastreliger kanagurta*) and Yellowtail scad (*Atule mate*)) and two demersal fish species (Forktail threadfin bream (*Nemipterus furcosus*) and Black pomfret (*Parastromateus niger*)), with total length range of 15.33 – 21.05 cm and weight range 44.42 – 115.17 g. These fish are some of the favourite commercial marine fish in peninsular Malaysia [5]. Forktail threadfin bream and Black pomfret were purchased from LKIM Endau, while Indian mackerel and Yellowtail scad were collected in the PTK Endau. Fish were traveled to the laboratory in the ice box and kept in the freeze at temperature of -20 ^{0}C microplastic extraction.

2.2 Microplastic extraction

The fish were thawed and rinsed with filtered distilled water before the microplastic extraction process. Moreover, total length and total weight were measured before the fish flesh was separated from the fish bones. Next, about 5-6 g of fish flesh were placed into a bottle and this step was repeated three times for each individual. In this digestion process, potassium hydroxide (10% KOH) was used (solution to organism ratio 10:1) [6]. The samples were then incubated at 60 °C for 24 hours using an oven [7]. Then, the solution was filtered using a glass filtration system and filter paper with a pore size of 1.2 µm and a diameter of 47 mm. The filter paper was dried in a glass petri dish in a 60 °C oven for 24–48 hours [8]. After fully dried, a stereo microscope was used to count the number, shape, size, and colour of microplastics on the filter paper, and a micro-FTIR was used to identify their type. Procedural blanks are utilized during laboratory work starting from dissection until identifying the microplastic process and all microplastics found in the same fish flesh as in the blank sample will not be counted.

2.3 Data analysis

Firstly, The Fulton condition factor (K) was calculated using following formula:

$$K = 100 \text{ x } \frac{W}{L^3} \qquad (1)$$

Where W is the weight of the fish species (g) and L is the length of the fish species (cm) [9]. Moreover, microplastic numbers in the fish flesh were calculated using the following formula:

$$Microplastic\ Number\ (pcs\ per\ gram) = \frac{MPs\ (pcs)}{Specimens\ weight\ (g)} \qquad (2)$$

Where the microplastic (pcs) is the microplastic total identified in a species from one sampling location, while specimens weight (g) is the sum of all fish flesh specimens of the species in one sampling location.

Statistical analyses were performed using the SPSS statistical analysis tool (version 27), with a significance level set at 0.05. The correlation between Fulton condition factor (K) with the amount of microplastics present in fish flesh. Moreover, the data of microplastic numbers were tested for the normality data using the Shapiro-Wilk test and the homogeneity of variance is referred to as the equality of variances. Then, after the rejection of parametric assumptions, a Kruskal-Wallis test was used to assess differences in the number of microplastics between species and between pelagic and demersal fish. Lastly, data of shape, size, type, and colour of microplastics were discussed descriptively.

3 Results and discussion

In this study, 120 fish specimen of Indian mackerel, Yellowtail scad, Forktail threadfin bream and Black pomfret were discovered with a range of total length 15.33 - 21.05 cm and total weight 44.42 - 115.17 g, which have a total of microplastics found are around 29 particles of all fish samples (Table 1).

Table 1. Biometrics parameter fish and microplastic contamination in fish.

Location	Indian mackerel	Yellowtail scad	Forktail threadfin bream	Black pomfret
Johor				
n Specimens	30	30	30	30
Mean Total length (cm)	19.65±0,95	15.33±1,43	21.05±1.00	16.32±0.90
Mean Weight (g)	87.87±11.85	44.42±13.65	115.17±16.84	85.72±12.41
Fish with MP (%)	30	23.33	6.67	20
Fish Flesh				
Specimens with MP (%)	10	10	2.22	7.78
Min - max MP in fish flesh (pcs/g)	0.000 – 0.125	0.000 – 0.132	0.000 – 0.061	0.000 – 0.125
Mean MP in fish flesh (pcs/g)	0.021	0.022	0.004	0.014

Yellowtail scad had the highest percentage of individuals with microplastics in their flesh, at 23.33 % with a mean of microplastic numbers of 0.022 particles per gram. Moreover, Indian mackerel followed by 30 % of individuals with a mean of 0.021 pcs/g. Furthermore, Black pomfret had the percentage of individuals at 20 % with a mean of microplastic numbers around 0.014. Forktail threadfin bream had the lowest rate of individuals at 6.67 % with microplastic numbers around 0.004 pcs/g.

Based on the statistical analysis, there is no significant difference in microplastic numbers between species ($p = 0.102$) (Table 2), however, there is a significant difference in microplastic numbers between pelagic and demersal fish ($p = 0.037$) (Table 3). Yellowtail scad and Indian mackerel are in pelagic fish [10], [11], while Forktail threadfin bream and Black pomfret include demersal fish [12], [13]. In current study, Pelagic fish has higher microplastic numbers than demersal fish. Yellowtail scad are usually quick swimmers and mostly consume tiny fish, crabs, cephalopods, copepods, decapod crustaceans, and prawns [14]. While Indian mackerel primarily feeds on plankton, encompassing phytoplankton, zooplankton, algae, and several other substances in differing quantities [15]. Moreover, pelagic fish, that consumes plankton for its whole life cycle, are susceptible to microplastic accumulation due to the size resemblance between microplastics and planktonic species [16]. In addition, [17] elucidated that filter feeders are generally more susceptible to the ingestion of microplastics than predatory species because of their non-selective feeding strategy.

Fulton condition factor (K) for all fish samples is generally higher than 1, which means that all fish samples are in good condition and healthy with a range of value of 1.03 - 2.28 (Table 2).

Table 2. Fulton condition factor (K) range of all fish samples in Johor Malaysia.

Location	Species	Fulton Condition Factor (K)
Johor	Indian mackerel	1.04 – 1.27
	Yellowtail scad	1.03 – 1.32

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	Forktail threadfin bream	1.06 – 1.43
	Black pomfret	1.75 – 2.28

The findings indicate a weak correlation between microplastic particles and the Fulton Condition Factor in Indian mackerel (Fig. 2). Merely 6.58% of the variation in the fish's condition can be accounted for by the amount of microplastic particles per gram, indicating a weak relationship. Moreover, this indicated that other variables may exert a more significant effect on the fish's health such as food availability and water quality.

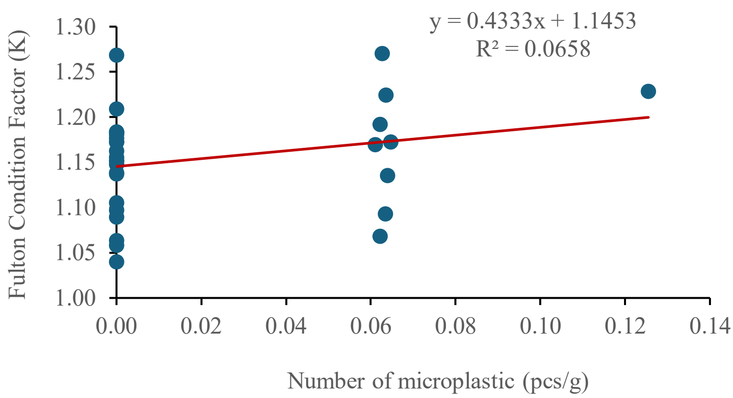

Fig. 2. Corelation of Fulton condition factor (K) with microplastic numbers in fish flesh of Indian mackerel.

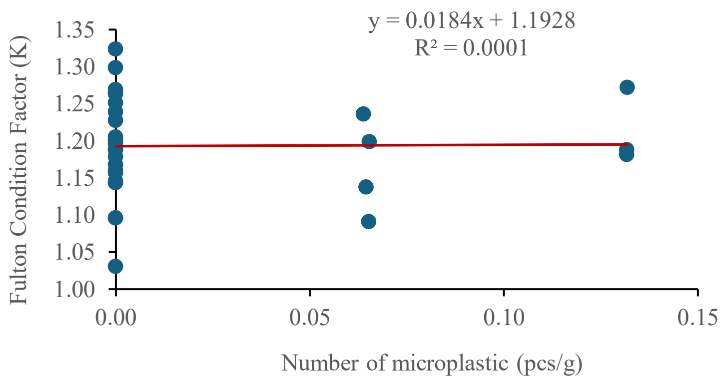

Fig. 3. Corelation of Fulton condition factor (K) with microplastic numbers in fish flesh of Yellowtail scad.

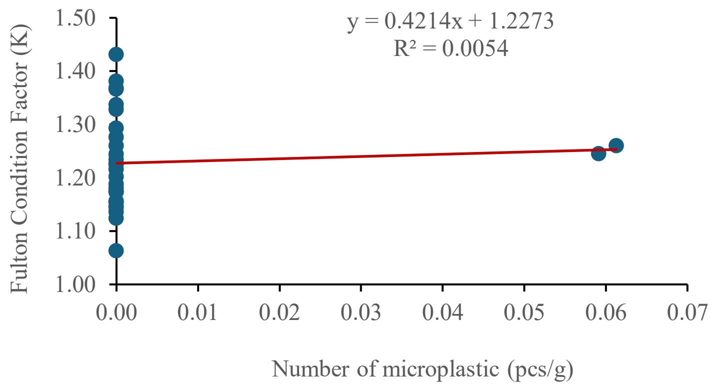

Fig. 4. Corelation of Fulton condition factor (K) with microplastic numbers in fish flesh of Forktail threadfin bream.

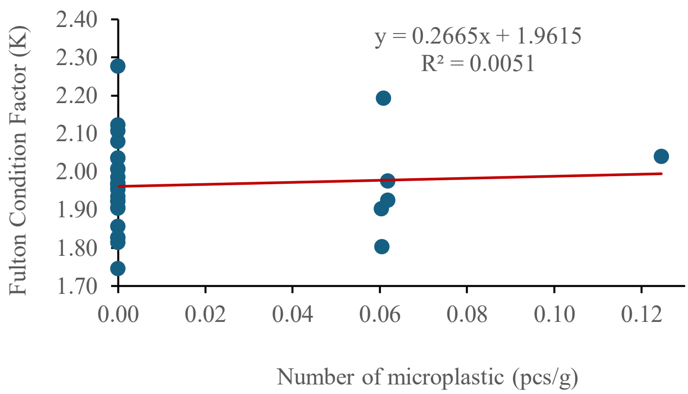

Fig. 5. Corelation of Fulton condition factor (K) with microplastic numbers in fish flesh of Black pomfret.

Yellowtail scad, Threadfin bream and Black pomfret had also weak correlation between the Fulton condition factor (K) and microplastic numbers in the fish flesh with R^2 value of 0.0001, 0.0054 and 0.0051, respectively. These findings illustrate that there is no significant effect of microplastic particle numbers on the fish's condition. The accumulation of microplastics in fish meat may remain below the safe limit, resulting in minimal effects on the fish. Furthermore, the condition of the seawater surrounding Johor continues to be favorable for the survival of these fish.

Size of microplastics predominant percentage found in fish flesh from all fish samples is < 200 µm, followed by 200 - 400 µm and > 400 µm as the smallest percentage (Fig. 6). Previous study by [18] found that almost all identified microplastic in muscles of both benthic and pelagic fish from northeast of Persian gulf are less than 300 µm. Moreover, the most common size of microplastic discovered in the muscle of *Gadus morhua* from western Norwegian waters was 32 - 100 µm [19]. Furthermore, [20] also identified that the most prevalent microplastic size found in three fish species from Blanakan, West Java, Indonesia was less than 300 microns. Smaller microplastics are presumed to have experienced greater plastic degradation, facilitating their accumulation in tissues and through trophic transfer.

Several comparable investigations from other marine environments, including the Mediterranean Sea and the Northeast Atlantic, found higher levels of microplastics in fish flesh compared to the current study. Barboza [21], who discovered three species of pelagic and demersal fish from the Northeast Atlantic, found that microplastic contamination in fish flesh with a size of 151 - 1500 µm varied between 0.4 and 0.7 pcs/g. Moreover, four fish species, pelagic and demersal fish, collected from the Mediterranean Sea found substantially greater microplastics in their flesh, ranging from 88,600 to 95,000 pcs/g with a much smaller size of <3 µm [22]. The discrepancies in microplastic reporting between research are mostly due to the significant variability in ingestion rates among different habitats, species, feeding behaviors, and geographical regions [23]. Additionally, even though the processes have yet to be understood, our findings, like those of previous research, support the concept that the small size of particles increases the accumulation of MPs in muscle.

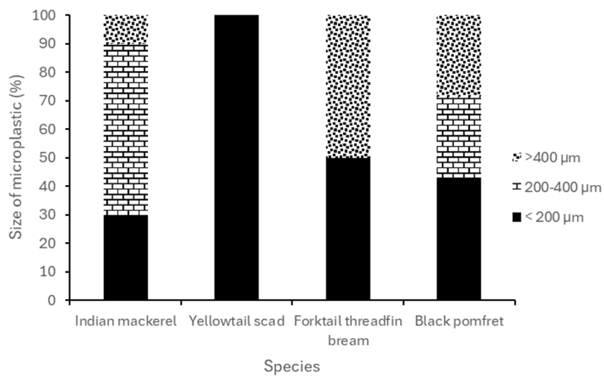

Fig. 6. Size of microplastics found in the fish flesh of commercial marine fish in Johor.

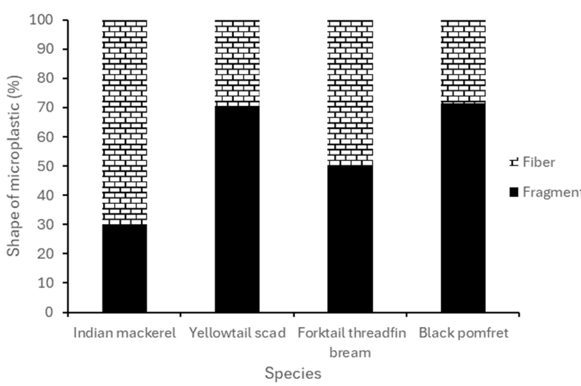

Fig. 7. Shape of microplastics found in the fish flesh of commercial marine fish in Johor.

The majority of microplastic shapes investigated in fish flesh of all fish samples were fragments with a range of 30 – 71.43 % of total microplastic, followed by fibres in a slightly low percentage with a range of 28.57 – 70 % of total microplastic found (Fig. 7). This finding is in line with the study by [7] that found the most commonly microplastics in the marine waters around Kuantan and Kuala nerus that also including to the east coast of peninsular Malaysia is fragments with a range of 66.1 % and 76.2 % of total microplastics, respectively [8]. Moreover, [11] studied on eleven commercial marine fish from fish market Seri Kembangan, Selangor also predominant contaminated by fragment in their excised organs and gills that reach to 67.4 %. Similarly, study on commercial marine fish from the seawater of northwest peninsular Malaysia revealed that the most frequent shape of microplastics found is fragments (49.5 %), fibres (41.9%) and pellet (7.6 %) [24]. This indicates that the level of fragmentation in the waters surrounding Peninsular Malaysia has increased over

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time. In addition, [25] added that the fragmentation of plastic trash, whether disposed of directly or indirectly, and the inadequate regulation of domestic and industrial waste disposal could contribute to the increased number of fragments in aquatic environments.

Polyamide is the predominant type of microplastic discovered in the four species of fish samples, particularly prevalent in Indian Mackerel, Forktail Threadfin Bream, and Black Pomfret (Fig. 8). Meanwhile, Yellowtail Scad contains the highest amount of Ethylene propylene diene monomer (EPDM). Moreover, the presence of Rayon, EPDM and other types of microplastic such as Polyvinyl alcohol (PVA), Melamine resin, and Polyacrylamide (PAM) are observed in lesser quantities throughout all species, exhibiting different proportions.

In previous study, Polyamide (PA) (Fig. 9a) also found as predominant type of microplastics in the surface marine waters around Terengganu and Kuantan Pahang [8], [26–28]. This type of microplastic is extensively utilized in fishing equipment (including nets, lines, and ropes), as well as in the textile [26, 29, 30] and automotive industries [31]. Moreover, as one of the main synthetic polymers utilized in staple textile fibers and materials for fisheries and aquaculture, PA may be responsible for the harm caused by textile fibers discharged into marine ecosystems through inadequately managed wastewater from washing machines and fishing gear [32]. In current study, the presence of PA in fish flesh is reasonable given the prevalence of fisheries activities and tourist attractions in several regions of Johor on the east coast, including Endau and Mersing. Furthermore, the compound demonstrates transmittance intensity at the following wavenumbers: 3282 cm^{-1} (N-H stretching), 2901 cm^{-1} (C-H stretching), 1639 cm^{-1} (medium N-H bending), 1558 cm^{-1} (N-H bending, C-N stretching), 1423 cm^{-1} (CH2 bending), 1311 cm^{-1} (CH2 bending) and 1228 cm^{-1} (NH bending, C-N stretching) [33]. In addition, according to ranking of plastic polymer types based on the hazard classifications of monomers by [34], polyamide (PA) is categorized at level III, with its monomer adipic acid known to induce eye irritation in humans.

[35] examined microplastic contamination in the gastrointestinal tracts of both wild and cage-cultured Asian sea bass (*Lates calcarifer*) and identified polyamide and polyvinyl alcohol (PVA) as the most prevalent type. PVA is extensively utilized for paper coatings and adhesives [36]. Furthermore, Rayon was identified in the gastrointestinal tract of Terapon jarbua from Sungai Besar, Kuala Selangor, Kuantan, and Mukah as the predominant microplastic type, constituting 83% of all microplastics detected [37]. This polymer is a semi-synthetic cellulosic fibre widely utilised in the manufacture of textiles, fabric upholstery, and sanitary products [38].

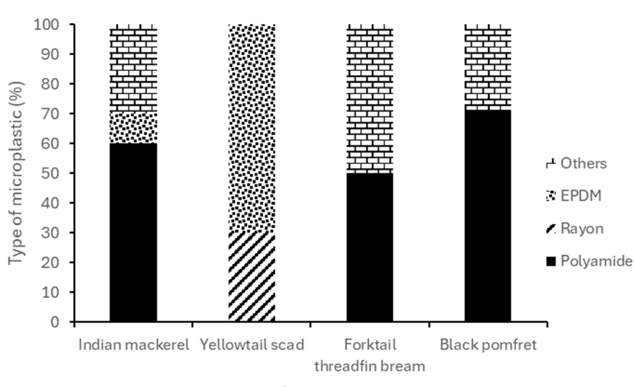

Fig. 8. Type of microplastics found in the fish flesh of commercial marine fish in Johor.

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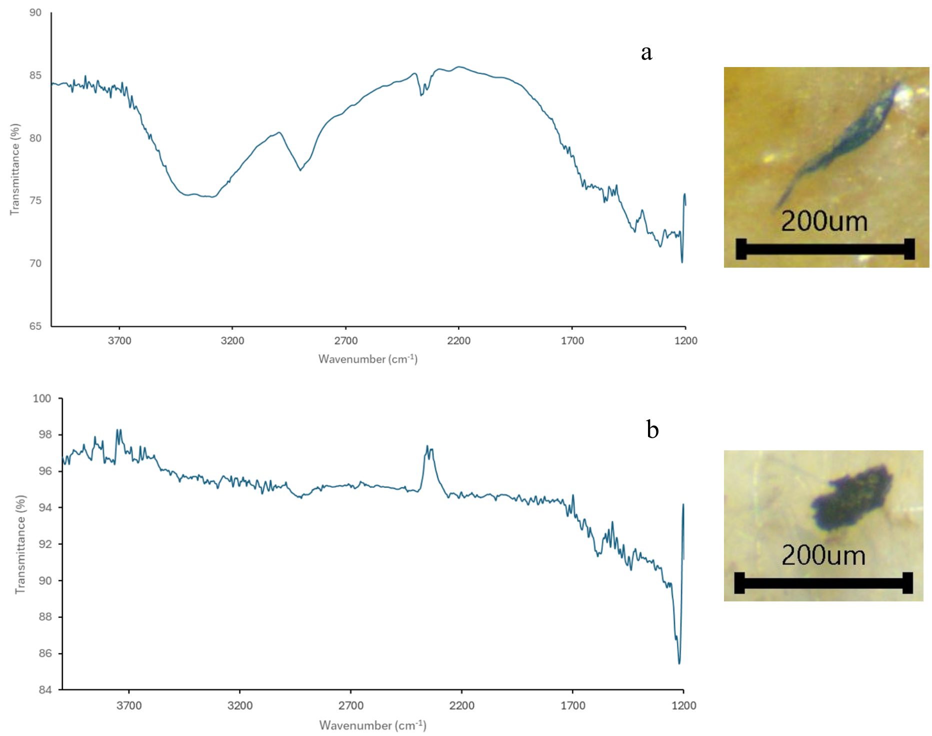

Fig. 9. The most prevalent of microplastic found in the fish flesh of commercial fish from Johor and their respective µ-FTIR spectra. a) Polyamide (PA); b) Ethylene propylene diene monomer (EPDM).

EPDM (Fig. 9b) is a synthetic rubber characterized by a chemical composition similar to polypropylene, containing ethylene and propylene, with the addition of additional monomers for enhanced double bonding, resulting in increased softness and elasticity [39]. The identification is characterized by peaks at around 2920 and 2850 cm^{-1}, corresponding to C–H stretching vibrations, and an absorption peak of the carbonyl group (C=C) near 1620 cm^{-1} attributed to diene groups in the structure [39, 40]. This rubber mostly contained in Rubber Type 3 (R3), utilised for many applications on land including seals, water hoses, and electrical insulation [41]. Moreover, the in vitro biocompatibility and cytotoxicity tests of EPDM rubbers on human normal cell lines demonstrated favourable safety properties. Thus, lots of this rubber is utilized in the production of rubber goods for diverse industrial and medical purposes [42, 43].

Microplastic polymers investigation in some species from five families of order Perciformes from the Ross Sea, Antarctica, could be divided into six types: polypropylene (PP), rayon, polyester (PES), polyacrylamide (PAM), ethylene vinyl acetate (EVA), and henequen, with PAM being the predominant polymer type with a range of 39 % from all microplastic detected. This polymer is extensively utilised in water treatment processes [44]. In this study, PAM is more prevalent in demersal fish flesh than in pelagic fish, perhaps due to its greater density compared to seawater, facilitating its descent into deeper waters. In addition, Melamine resin is a kind of plastic classified within the thermosets category, which consists of polymers that experience chemical transformations upon heating. These polymers cannot be re-melted and re-shaped after heating and forming [45].

The prevalent colour of microplastic distributed throughout all four species is black, with a particularly high prevalence in Indian Mackerel (Fig. 10). Blue colour ranks as the second most prevalent colour across all species. Although less common overall, red microplastics are more noticeable in Black Pomfret than in other species. Although present, the others group such as chocolate and transparent has the lowest proportion of microplastics among all species.

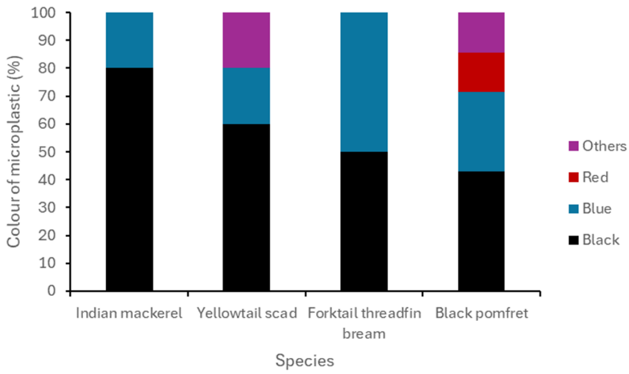

Fig. 10. Colour of microplastics found in the fish flesh of commercial marine fish in Johor.

Black microplastics are the ones most commonly found in most global microplastic investigations. Prior research by [35] revealed that black microplastics are the most prevalent type found in both cage-cultured and wild *Lates calcarifer* in the Setiu wetlands of Terengganu. Furthermore, 71% of microplastics identified in various tissues, including the muscles of pelagic and demersal fish from the Persian Gulf, were black or grey [46]. Additionally, an examination of the muscles of 17 fish from the mangrove estuary of Bangladesh revealed that black microplastics constituted 50% of the observed microplastics [47].

4 Conclusion

This study highlights the presence of microplastic contamination in the flesh of commercial marine fish from Johor, Peninsular Malaysia, with pelagic fish showing higher microplastic accumulation than demersal fish. The findings also reveal that microplastic contamination does not significantly affect the condition of the fish, as indicated by the weak correlation with the Fulton condition factor. Predominantly black microplastic fragments smaller than 200 µm, primarily composed of PA and EPDM polymers, were identified in the fish flesh. These results underscore the importance of further research and regular monitoring on microplastic pollution in commercial marine fish, as well as its ecological impacts and its risk to human health in Peninsular Malaysia, especially in Johor.

Acknowledgements

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