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RESEARCH ARTICLE

MICROPLASTICS IN THE SHORTFIN SCAD *DECAPTERUS MACROSOMA* FROM THREE SELECTED WET MARKETS, SABAH

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Abstract. Microplastics (MPs) have become a major concern as a source of environmental pollutants in marine ecosystems. Fish such as shortfin scad are consumed daily, particularly in Sabah and an important protein source. It is also being used as feed for marine finfish such as grouper. This study aimed to characterize the types of MPs isolated from *Decapterus macrosoma* from three selected wet markets at Sabah. A total of 21 specimens of fish were collected from three selected wet markets, respectively, around Kota Kinabalu. The fish were dissected, and their gastrointestinal tract (GIT) and flesh were separated for digestion. The exudates were filtered several times using filter paper. MPs isolated were characterized using microscopes and the polymers were identified using Fourier Transform infrared spectroscopy (FTIR). The MPs were measured using ImageJ software. The total MPs collected from both organs was 1541. Pasar Ikan Tuaran specimens recorded the most MPs in flesh (n=263) and GIT (n=422). The most abundant shape was fiber (n=1406, $p<0.05$) and the most significant color was black (n=866, $p<0.05$). The polymers that were detected from both GIT and flesh were polyamide (n=39), followed by polycarbonate (n=21) and poly(methyl methacrylate) (n=11). In conclusion, the MPs were found in the flesh and GIT of *D. macrosoma* with the majority from polycarbonate and PMMA. These results show concern about seafood safety in Malaysia with regard to Sabah.

Keywords: *Decapterus macrosoma*, seafood safety, marine finfish, microplastics.

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1. INTRODUCTION

Plastic usage has caused great convenience in our daily lives but also comes with ill consequences. Studies estimate that from 4.8 million metric tons to 12.7 million metric tons of plastics are produced yearly [1]. Microplastics (MPs) are known as plastic with a size ranging from 5 mm down to 1 µm [2]. Recently, there has been a debate about the dangerous effects of MPs coming from the environment due to the extensive use of goods made from polymers. Microplastics (MPs) move through the food chain as smaller marine organisms ingest them, passing them to larger predators and eventually to humans through seafood consumption. Studies have found MPs in edible species like shellfish and crustaceans, raising concerns about human exposure [3,4].

MPs ingested by the fish via their digestive tract will accumulate in the GIT. However, there are a lack of studies on the MPs accumulation in the flesh. Previous studies have shown evidence of MPs presence in seafood, such as polyamide (PA), polycarbonate (PC), poly(methyl methacrylate) (PMMA), polyvinyl chloride (PVC) and nitrile [5,6] and all studies targeted the intestine as target organ sample. MPs ingestion can harm fish physically and mechanically and obstruct their digestive tracts. Moreover, it offers a potential route for the entry of hazardous compounds into the aquatic food web, such as plastic additives, toxic chemicals absorbed from ambient matrices, and pathogenic bacteria inhabiting the plastics.

Fish is a cheap source of protein from seafood. It is the main protein source, particularly for the coastal communities. They are also being made as feed for bigger marine finfish. The impact of MPs does not stop with small fish. Bigger fish that prey on these smaller fish are exposed to the same harmful effects. The ingestion of MPs-laden fish may cause detrimental physiological changes in larger predators, affecting their growth, behavior, and overall health [3]. Additionally, the transfer of MPs up the food chain raises concerns about the potential risks to human health, as larger fish species consumed by humans may also harbor these pollutants. Understanding the implications of MPs in small fish and their effect on larger predators is crucial for addressing the broader environmental and ecological risks associated with plastic pollution.

Hence, this study aimed to evaluate the MPs in the shortfin scads *Decapterus macrosoma*'s flesh and gastrointestinal tract (GIT) from three selected wet markets, which were Pasar Ikan Tuaran, Donggongon Fish Market, Sabah Fish Marketing Sdn. Bhd. (SAFMA) in Kota Kinabalu, Sabah. The types of the polymers were detected using FTIR spectroscopy from both organs. Furthermore, this paper also discusses the impact of reckless plastic use, emphasizing its convenience and environmental consequences. It underscores the issue of MPs contamination in seafood, particularly fish, and the potential risks to human health and marine ecosystems. The study seeks to assess MPs' implications for food safety and environmental health, providing more insight into this environmental toxicity to the public and worldwide awareness.

2. MATERIALS AND METHODS

2.1 Sample Preparation

A total of 21 dead fish (*D. macrosoma*) with lengths ranging from 15 to 19 cm and weighing from 46 to 60 grams were bought from selected wet markets; the Pasar Ikan Tuaran (6.1794 °N, 116.2330 ºE), Donggongon Fish Market (5.9121 °N, 116.1018 °E) and Sabah Fish Marketing Sdn. Bhd. (SAFMA) (5.9827 °N, 116.0716 °E) respectively; and were used for the evaluation [7]. Before thawing, each of the fish was re-rinsed with distilled water. All the fish were measured for their body length, height, weight and width using a measuring tape and analytical balance to the nearest 0.01 g. The fish was dissected for the GIT and the rest of the flesh was studied for its MPs content separately before being evaluated [6]. All beakers were filled with 95 % ethanol solution with a ratio of 1:4 for 1 hour to clean the flesh and GIT before further identification. Then, the flesh and GIT were stored separately in new empty and clean glass beakers; all samples were labelled accordingly before further analysis [8,9].

2.2 Isolation of Microplastics

A total of 10 % NaOH (w/v) (with a ratio of 1:6) was added to each of the 250 mL glass beakers containing 30-80 g of fish's flesh and incubated for 72 hours at 40 °C. A total of 10 % NaOH (w/v) (with a ratio of 1:6 of organ and volume of NaOH) was added to each of the 100 mL glass beakers containing 0.50-5.60 g of fish's intestine and incubated for 72 hours at 40 °C, this method was adapted from Constant et al. [8], with modification. The digestates were filtered with vacuum filter paper (pore size 11 µm). The filtered yield was soaked in 10 to 15 mL of 4.4 M sodium iodide (NaI) (density 15 g/mL), sonicated at 50 Hz for 5 mins, and agitated on an orbital shaker for 5 mins at 200 rpm to isolate the high-density particles [8]. The solution was centrifuged at 500×g for 2 mins. The MPs solution was vacuum-filtered through a glass filter (pore size 1.2 µm). These steps were repeated thrice to ensure the complete isolation of MPs [10]. A total of 10 % NaOH (with a ratio of 1:6 of flesh and volume of NaOH) was added to each of the 250 mL glass beakers containing 30-80 g of fish's flesh and incubated for 72 hours at 40 °C. A total of 10 % NaOH (with a ratio of 1:6 of organ and volume of NaOH) was added to each of the 100 mL glass beakers containing 0.50-5.60 g of fish's intestine and incubated for 72 hours at 40 °C. The digestates were filtered with vacuum filter paper (pore size 11 µm). The filtered yield was soaked in 10 to 15 mL of 4.4 M sodium iodide (NaI) (density 15 g/mL), sonicated at 50 Hz for 5 mins, and agitated on an orbital shaker for 5 mins at 200 rpm to isolate the high-density particles. The solution was centrifuged at 500×g for 2 mins. The MPs solution was vacuum-filtered through a glass filter (pore size 1.2 µm). These steps were repeated thrice to ensure the complete isolation of MPs [11].

2.3 Morphological Observation

The MPs were observed through a compound microscope (Olympus CX23) to identify the length, colour, and shape, such as the threadlike, fragment, and type of MPs. ImageJ software was used to measure the size of each MPs. A needle was heated on a flame and was put onto the presumptive MPs to check whether there were changes in the shape of the sample. Plastic MPs was curl and crump and this method was used to differentiate them from other types of debris. The identified MPs were placed on a glass petri dish that was covered with filter paper [8].

2.4 FTIR Analysis

The FTIR analysis to identify the plastics components was performed using a Thermo Fisher Scientific NICOLET iS50 FTIR Spectrometer. The instrument was equipped with a deuterated triglycine sulfate (DTGS)-potassium bromide (KBr) detector and XT-KBR beam splitter. A background scan was collected before each sample on a clean slide and recorded as blank using attenuated total reflection (ATR) imaging, using 32 co-added scans in the mid-IR range of 4000-650 cm^{-1}.

2.5 Quality Assurance and Quality Control

The MPs analysis was conducted in well-covered and protective places from open air to avoid contamination. Equipment, synthetic clothing, and tools made from plastics were prohibited during the process. The storage equipment was plastic-free in composition and location, and filtered solutions or solids were sealed in glass containers/bottles [12].

2.6 Statistical Analysis

Statistical analyses were analyzed using the IBM Statistical Package for Social Science (SPSS) version 29.0. One-way analysis of variance (ANOVA) was utilized with the Bonferroni Test of post-hoc analysis applied when the data had equal variance. Statistical significance was set at $p < 0.050$.

3. RESULTS AND DISCUSSION

3.1 Microplastics Quantification in Fish

Based on Figure 1, the total MPs in flesh and GIT of all samples from the three markets in Sabah, Malaysia were 1541. The highest number of MPs were present in the GIT with 889 particles, while 652 particles were in the flesh. The total number of MPs in all markets was recorded with Pasar Ikan Tuaran having 263 particles in the flesh and 422 particles in GIT respectively, while Donggongon Fish Market recorded a total of 194 particles in the flesh and 254 particles in GIT. As for the SAFMA, the number of MPs was 195 in the flesh and 213 in the GIT. The whole *D. macrosoma* that was used for the identification in this study contained 652 MPs in flesh whilst 889 MPs in GIT.

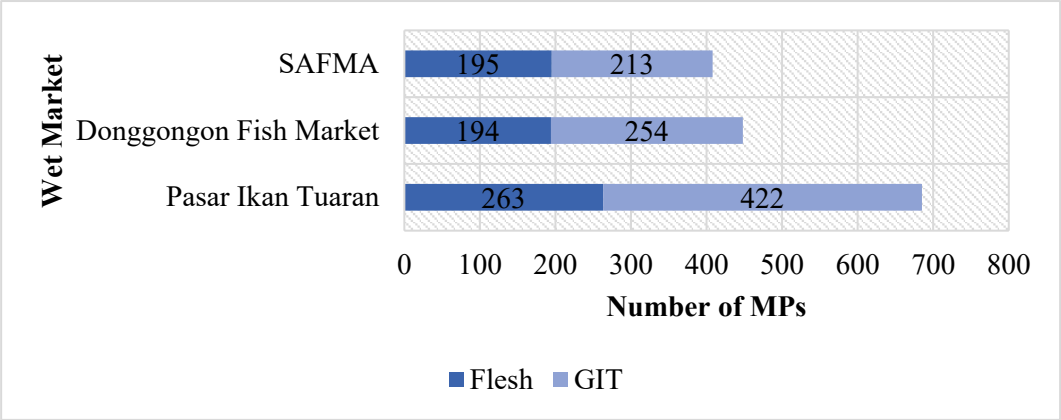

Figure 1: The total number MPs detected from the flesh and GIT of the *D. macrosoma* (n=21 from each wet market respectively)

3.2 Microplastics Characterization in Fish

Figures 2 and 3 show the MPs isolated from flesh and GIT in various sizes, shapes and colors. All the reported sizes were smaller than 5 mm. The size of the MPs was measured by using ImageJ software and length (L) for fibers, height (H) and width (W) for fragments and films.

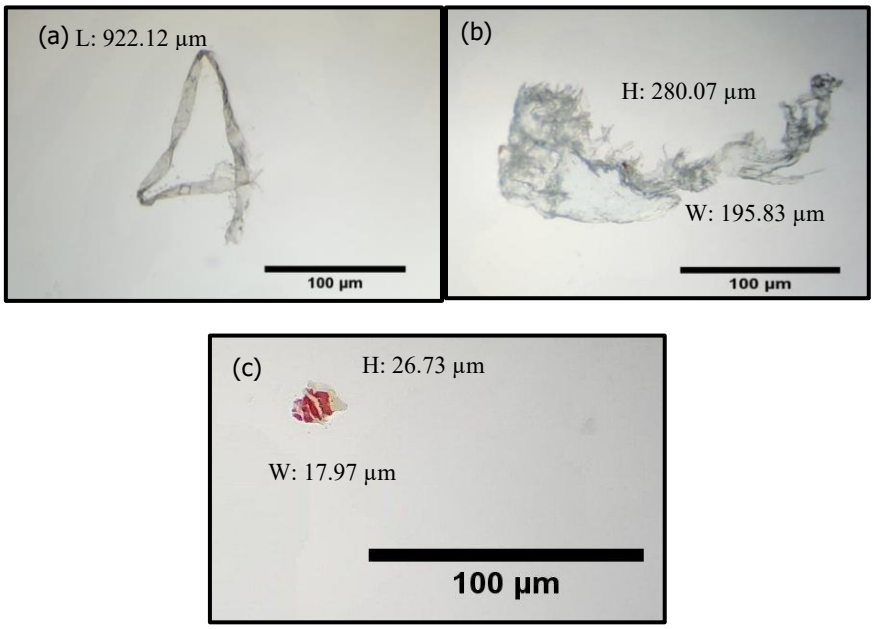

Figure 2: The size of MPs from fish flesh (a) fiber-shape, (b) fragment-shape and (c) film-shape

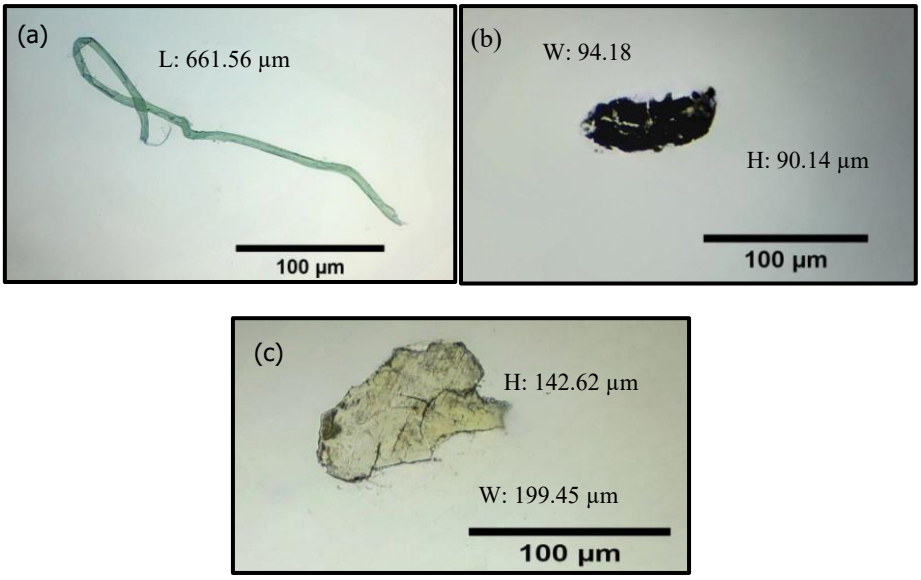

Figure 3: The size of MPs from fish GIT (a) fiber-shape (b) fragment-shape and (c) film-shape

The shapes of MPs were categorized into fiber, fragment, and film. Notably, in Table 1, the fiber MPs from fish flesh dominates in terms of abundance. The samples from Pasar Ikan Tuaran have the most fiber-shape (246) MPs that were identified from 21 shortfin scad, followed by Donggongon Fish Market (158) and SAFMA (145). Significant differences were observed in the fiber-shaped microplastics present in flesh samples between Pasar Ikan Tuaran samples and both Donggongon Fish Market samples ($p = 0.001$) and SAFMA samples ($p = 0.001$). In flesh samples, both fragment and film shapes exhibited significant differences between Donggongon Fish Market and Pasar Ikan Tuaran (fragments: $p = 0.001$; film: $p = 0.001$), as well as between Donggongon Fish Market and SAFMA (fragments: $p = 0.001$; film: $p = 0.010$).

Table 1: The percentage of microplastics shape detected from shortfin scads flesh from pasar ikan Tuaran, Donggongon Fish Market and SAFMA

Wet Market	Shape		
	Fiber (%)	Fragment (%)	Film (%)
Pasar Ikan Tuaran (n =21)	93.54 ± 3.77^{a}	6.08 ± 1.41^{a}	0.38 ± 0.22^{a}
Donggongon (n = 21)	81.44 ± 3.75^{b}	8.25 ± 0.83^{b}	10.31 ± 0.97^{b}
SAFMA (n =21)	74.36 ± 2.68^{b}	23.08 ± 1.68^{a}	2.56 ± 0.44^{a}

* Values are shown as Mean ± SD. Percentage within the same column (for each parameter) carrying different superscripts are significantly different at $p < 0.050$. The symbols a, b and c show the significance if not presented in the same column boxes.

The high prevalence of fibers was attributed to their ease of formation during the deterioration phase. Throughout all of the three selected wet markets, both fragments and films shapes appeared to be fewer in numbers compared to the fiber shape. Based on previous paper, a huge number of fibers located in their samples disregard places [8]. Among the three markets, SAFMA samples exhibited the highest number of fragment-shaped MPs, with 45 unit. In contrast, Donggongon Fish Market had the highest number of MPs in film shape, totaling 20. Fragments of about 16 were discovered in both Pasar Ikan Tuaran and Donggongon Fish Market samples. Five films were detected in SAFMA fish and only one film was from Pasar Ikan Tuaran samples.

In the GIT samples, based on Table 2, the highest number of fibers were detected in Pasar Ikan Tuaran, totaling 382, followed by 215 in Donggongon Fish Market, with SAFMA having the lowest count of 157. In the GIT, the presence of fiber-shaped MPs was significant only in Pasar Ikan Tuaran samples compared to both Donggongon Fish Market samples ($p = 0.001$) and SAFMA samples ($p = 0.001$). However, no significant differences were observed in GIT between Pasar Ikan Tuaran and SAFMA samples for both fragment and film shapes. The contamination of organisms studied by MPs does not exhibit a consistent pattern; instead, it can be influenced by various contributing factors such as habitat preference, feeding habits, and the size of the organisms [13]. The second highest number of shapes reported in GIT was fragment, and the lowest number of shapes was film. In SAFMA, the fragment recorded was 44, and the film was 12. As for Pasar Ikan Tuaran, 33 fragments with 7 films were recorded, and lastly, on Donggongon Fish Market samples, it was discovered that 24 fragments and 15 films were identified throughout the observation.

Table 2: The percentage of microplastics shape detected from shortfin scad GIT from pasar ikan Tuaran, Donggongon Fish Market and SAFMA

Wet Market	Shape		
	Fibre (%)	Fragment (%)	Film (%)
Pasar Ikan Tuaran (n =21)	90.52 ± 5.57a	7.82 ± 1.50a	1.66 ± 0.80a
Donggongon (n = 21)	84.65 ± 3.85b	9.45 ± 2.20a	5.91 ± 0.85a
SAFMA (n =21)	73.71 ± 2.84b	20.66 ± 2.14a	5.63 ± 0.81a

* Values are shown as Mean ± SD. Percentage within the same column (for each parameter) carrying different superscripts are significantly different at $p < 0.050$. The symbols a, b and c shows the significance if not presented in the same column boxes.

In previous studies by Barboza et al. [10], a total of 368 MPs were isolated from species such as *Dicentrarchus labrax*, *Trachurus trachurus*, and *Scomber colias*. Additionally, 296 MPs were found in species, including *Leporacarus gibbus*, *Mugil cephalus, Plectropomus leopardus*, and *Upeneicythys lineatus* [11]. Based on the previous study by Kandeyaya et. al. [12] at the West Coast Sri Lanka, they suggested that the plastic contamination might come from increasing fishing activities. The scientific explanation of the MPs colonization of the ocean has not been proven, but it does remark that these emerging MPs are currently severe, and their numbers may increase day by day.

This study also investigated the colors of MPs (Table 3), to identify potential differences in the predominant colors that may have migrated into the body wall of the fish. The tabulated results indicated that black was the most frequently sorted color among the three wet markets, while orange and yellow were the least detected colors, with only two colors present. Significant differences were observed in the pink microplastics (MPs) between the Pasar Ikan Tuaran and Donggongon samples ($p = 0.001$), and a similar significant difference was observed between the Donggongon Fish Market and SAFMA samples ($p = 0.001$). Furthermore, blue color MPs was shown to be significant ($p = 0.038$) between Pasar Ikan Tuaran samples and Donggongon Fish Market samples.

Table 3: The percentage of microplastics detected from shortfin scads flesh from three selected wet markets based on their color

Wet Market	Color						
	Black (%)	Pink (%)	Blue (%)	Red (%)	Brown (%)	White (%)	Others (%)
Pasar Ikan Tuaran (n = 21)	62.36 ± 3.03^{a}	5.70 ± 0.78^{a}	11.03 ± 1.53^{a}	4.56 ± 0.75^{ab}	6.08 ± 1.00^{ab}	2.66 ± 0.86^{a}	7.6 ± 1.65^{a}
Donggongon Fish Market (n = 21)	52.82 ± 2.28^{b}	5.64 ± 0.60^{b}	12.82 ± 1.33^{b}	3.59 ± 0.48^{a}	6.67 ± 0.86^{a}	10.26 ± 0.97^{a}	7.69 ± 1.33^{a}
SAFMA (n = 21)	61.03 ± 2.24^{c}	5.13 ± 0.60^{a}	8.21 ± 0.89^{ab}	4.10 ± 0.80^{b}	11.28 ± 1.02^{b}	4.62 ± 0.68^{a}	5.64 ± 1.12^{a}

*Values are shown as Mean ± SD. Percentage within the same column (for each parameter) carrying different superscripts are significantly different at $p < 0.050$. The symbols a, b and c show the significance if not presented in the same column boxes.

Similarly, the results in Table 4 for the gastrointestinal GIT samples of all 63 fish showed a repetitive pattern in the detection of MPs colors, with the highest and lowest abundances mirroring those observed in the flesh samples. Pasar Ikan Tuaran samples showed significant differences compared to both Donggongon Fish Market and SAFMA samples for black color ($p = 0.001$) and brown color ($p = 0.030$). The black colors of MPs suggested that many fishing activities were conducted at the ocean [8]. Fishing items such as nets and ropes are commonly manufactured in black color. Additionally, black-colored MPs can also originate from tire wear and tear, as well as the laundering of synthetic garments. In a study conducted by Wootton et al. [13], it was found that marine animals such as *Scylla serrata* (1.8 MPs/g), *Penaeus monodon* (1.7 MPs/g), and *Katsuwonus pelamis* (1.42 MPs/g) exhibit a targeted approach in their feeding habits, suggesting they can selectively consume specific types of MPs resembling prey items.

Table 4: The percentage of microplastics detected from shortfin scads GIT from three selected wet markets based on their color

Wet Market	Color						
	Black (%)	Pink (%)	Blue (%)	Red (%)	Brown (%)	White (%)	Others (%)
Pasar Ikan Tuaran (n = 21)	61.61 ± 4.42^{a}	3.08 ± 0.92^{a}	8.77 ± 1.37^{a}	7.82 ± 1.33^{a}	9.48 ± 1.45^{a}	2.84 ± 0.98^{a}	6.4 ± 1.42^{a}
Donggongon Fish Market (n = 21)	63.08 ± 4.20^{b}	4.10 ± 0.59^{a}	15.90 ± 1.54^{a}	9.23 ± 0.91^{a}	9.74 ± 1.00^{b}	13.85 ± 1.10^{a}	14.36 ± 1.63^{a}
SAFMA (n = 21)	45.54 ± 3.11^{b}	7.98 ± 0.87^{a}	11.74 ± 1.29^{a}	9.39 ± 0.92^{a}	8.92 ± 1.18^{b}	10.33 ± 0.80^{a}	6.11 ± 1.61^{a}

* Values are shown as Mean ± SD. Percentage within the same column (for each parameter) carrying different superscripts are significantly different at $p < 0.050$. The symbols a, b and c show the significance if not presented in the same column boxes.

3.3 FTIR Analysis

The MPs The FTIR result was taken by using FTIR in the mid-IR range of 4000 to 650 cm^{-1}. The MPs were analyzed using the specific reading numbers shown on the graph and compared to the nearest readings of each polymer peak that represents the polymer functional groups. The functional groups were made up from chemical compositions which are the long chains of mixed monomers together with the present of extra chemical functional elements.

In Figure 4, the spectrum of PA reveals the presence of specific functional groups at various wavenumbers: 3004.31, 2853.44, 1587.23, 1438.90, 1292.71 and 1135.78 cm^{-1}. Each of the readings listed has a specific group of polymer functional groups, such as 3004.31 cm^{-1} and 2853.44 cm^{-1}, which are CH stretches. Notably, the CH_2 bend exhibits the highest number of detected peaks, indicating the prevalence of functional groups within the PA polymer. Second, CH_2 bend readings at 1587.23, 1438.90 and 1135.78 cm^{-1}. The last one, 1292.71 cm^{-1} represents NH Bend and C-N Stretch together. PA were used for its high in durability, materials that made from PA are synthetic textiles, fishing nets and fishing braid line, thus, this PA were highly detected in this our samples, the contamination from PA is inevitable, due to the fishing activities, here in Sabah, the fishing activities by the locals are ubiquitous. Moreover, the fish caught can be assumed by huge fishing nets that was set up at the sea. It is not possible that this net is responsible of the contamination in the *D. macrosoma.*

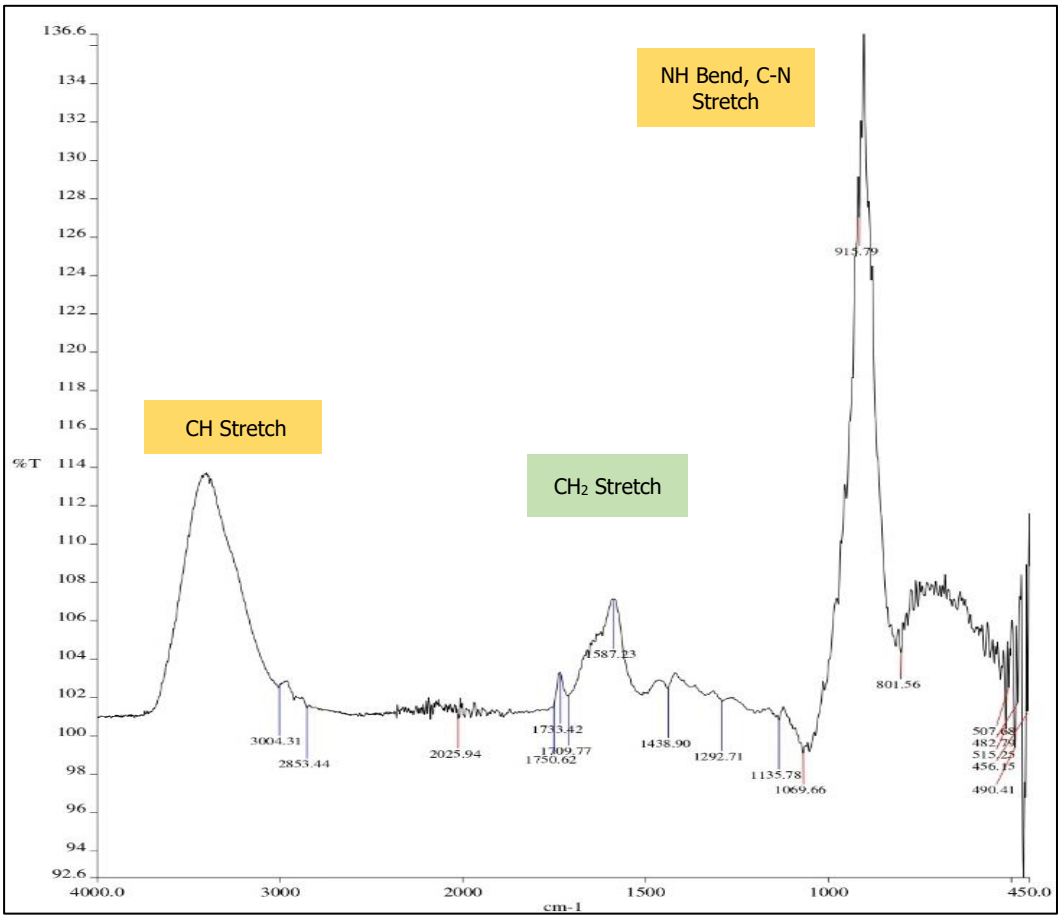

Figure 4: The FTIR spectrum of PA polymer from a fish GIT

In Figure 5, polycarbonate-microplastic (PC-MP) isolated from fish flesh shows distinct functional groups at prominent peaks: C-O stretch (1419.92 cm^{-1}), aromatic ring stretch (1720.25.67 cm^{-1}), C=O stretch (1783.92 cm^{-1}) and aromatic CH out of plane bend (796.14 cm^{-1}). PC was one of the highest numbers detected from the fish. The leached from the waste plastics bottles by natural or human activities such as sunlight or personal cares product may end up in to the sea and unknowingly ingested by the fish.

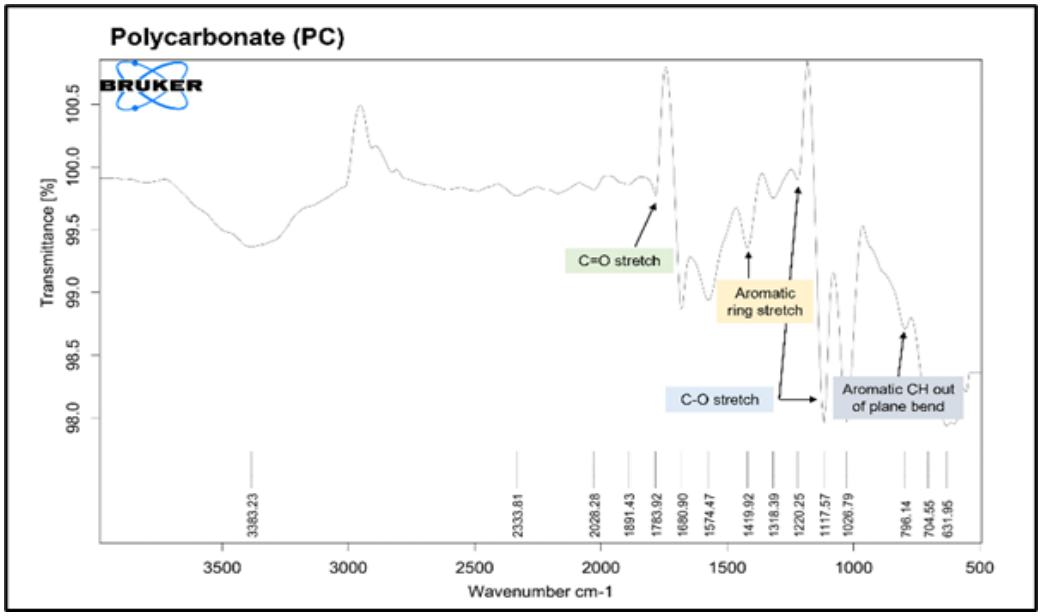

Figure 5: The FTIR spectrum of PC polymer from a fish GIT

In Figure 6, poly(methyl methacrylate) microplastics (PMMA-MP) isolated from fish flesh exhibits functional groups at prominent peaks: CH stretch (2907.08 cm^{-1}), C=O stretch (1710.82 cm^{-1}), CH bend (842.90 cm^{-1}), C=O stretch (1246.39 cm^{-1} and 1076.88 cm^{-1}) and C=O bend (633.25 cm^{-1}). All samples were analyzed using FTIR, and the detection of polymers was based on the identified functional groups mentioned above. As for the PMMA, commonly known as acrylic glass, finds extensive use in the automotive and maritime transport industries due to its exceptional impact and ultraviolet resistance properties. In addition to being a prominent fishing area, Kota Kinabalu and Kuala Selangor are situated along one of the world's busiest shipping lanes, where anthropogenic activities are prevalent at a higher rate [4].

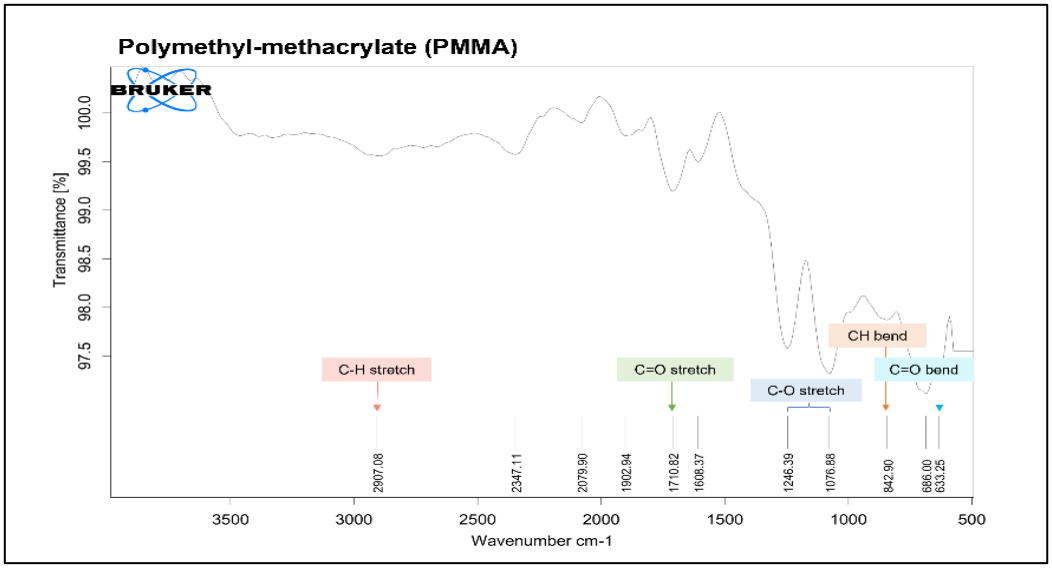

Figure 6: The FTIR spectrum of PMMA polymer from a fish flesh

In the same study by Kandeyaya et al. [11], from fish (*Symphodus* spp., *Diplodus* spp., and *Sardina* sp.) in the Mediterranean Sea, the samples confirmed the presence of PETE (45 %), PA (36 %), acrylic (9 %), and polypropylene (9 %). In another study, the highest abundance of polymers of polyolefin was detected in *Leporacarus gibbus*, *Mugil cephalus*, *Plectropomus leopardus*, and *Upeneicythys lineatus* from Australia and Fiji river sites [10]. In addition to PA, PC and PMMA, there were also other polymers detected from both flesh and GIT of *D. mascrosoma* which were EVA, Nitrile, LDPE, PS and ABS. Interestingly, PETE and PVC were present in flesh samples, which was not detected in GIT samples.

4. CONCLUSIONS

The results of this study showed the presence of MPs in the flesh and GIT of *D. macrosoma* and that they were potentially consumed by bigger fish (finfish) and humans. The total number of MPs isolated from both flesh and GIT was 1541; Pasar Ikan Tuaran recorded the highest number of MPs in the GIT (47.46 %) and flesh (40.33 %). From the FTIR analysis, the polymers that were detected from both GIT and flesh were the PA (39) followed by PC (21) and PMMA (11). The MPs of fiber-shaped and black color comprised the majority of MPs accumulated in both the *D. macrosoma* flesh and GIT. Pasar Ikan Tuaran shows significant amounts of MPs in both their flesh and GIT. This study has employed a strict quality control to ensure there was no contamination. The results from this study served as preliminary data to prove the presence of MPs contamination in seafood, particularly fish. The MPs presence might cause toxicity effects on the fish itself and eventually might case harmful effects towards consumer.

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Author Contributions

Nurzafirah Mazlan and Rossita Shapawi contributed towards the grant acquirement. Muhammad Nor Afdall Nazahuddin, Sarah Syazwani, Nur Nashrah collected the data and analyzed. Siti Marwanis Anua analyzed the data. Abentin Estim and Muhammad Dawood Shah contribute to the research design and supervision. All authors contributed toward data analysis and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

This research was approved by the Animal Ethics Committee of Universiti Malaysia Sabah with ethics code: AEC 0022/2022.

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