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# Occurrence, distribution and characteristics of microplastics in gastrointestinal tract and gills of commercial marine fish from Malaysia

Norhazwani Jaafar [a], Ahmad Azfaralariff [a], Syafiq M. Musa [b], Mazlan Mohamed [c], Abdul Hafidz Yusoff [c], Azwan Mat Lazim [a,*]

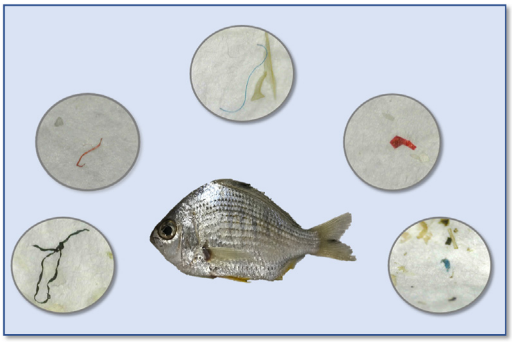
[a] *Department of Chemical Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*
[b] *Department of Earth Sciences and Environment, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia*
[c] *Advanced Material Research Cluster (AMRC) Faculty of Bioengineering and Technology (FBET), Universiti Malaysia Kelantan Kampus Jeli, Locked Bag 100, 17600 Jeli, Kelantan, Malaysia*

## HIGHLIGHTS

- MPs were detected in gastrointestinal tract and gills of commercial marine fish.
- High incident of MPs found in fishes from coastal water close to urban area.
- Larger size MPs were dominant in gills, while smaller in GIT.
- Heavy metals namely Cr and Fe were detected on coloured MPs.

## GRAPHICAL ABSTRACT

Microplastics in commercial marine fishes from Malaysia waters



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## ABSTRACT

Microplastics are tiny plastic particles with size below 5 mm, prevalence in marine environments and the occurrence have been reported in commercial marine fish worldwide. Microplastics' abilities to absorb various marine contaminants raised considerable concern on their role as a vector to spread harmful pollutants to the alienated environment. This study focussed on the occurrence of microplastics in gastrointestinal tract (GIT) and gills of 158 fishes across 16 species from two locations in Malaysia coastal waters. Microplastics were detected approximately 86% in the GIT and 92% in the gills of examined fish. High incident of microplastics was detected in fishes from the area that is close to an urban area with average microplastics incident reaching up to 9.88 plastics items/individuals. Meanwhile, only 5.17 microplastics per individual were recorded in fishes from a less urbanised area. Isolated microplastics comprised 80.2% of fibres, 17.7% of fragments and the remaining was derived from filaments (3.1%). Infrared and Raman spectroscopy analysis of selected microplastics revealed the chemical composition of microplastics which comprised of polyethene (PE), polypropylene (PP), acrylonitrile butadiene styrene (ABS), polystyrene (PS) and polyethylene terephthalates (PET). FESEM images indicate, different surface characteristics of microplastics as a result of environmental exposure. Further, elemental analysis using EDX for green PE fragments showed the uneven distribution of chromium (Cr) and iron (Fe) on the surface, suggesting the adherence of heavy metals on the surface of microplastics. Overall findings indicate the widespread distribution of microplastics in commercial marine fishes from Malaysia waters and could potentially lead to human exposure through fish consumption.

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* Corresponding author.
*E-mail address:* azwanlazim@ukm.edu.my (A.M. Lazim).

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## 1. Introduction

Global consumption of fish per capita has risen drastically over the past five decades, growing from 9 kg in 1961, passing more than 20 kg in 2018 with an average annual rate of 3.1% for the same period (FAO, 2020). Developing countries experience rapid growth of fish consumption from 5.2 kg in 1960 to 19.4 kg in 2017, primarily influenced by the rapid population and urbanization (Delgado et al., 2003). Malaysia is one of the world's top fish consumers, estimated at 59 kg/head/year in 2016 (FOA, 2018; Goh, 2018). The growing demand over fish protein is matched by a rising public consciousness of the essential nutritional components such as omega-3 and nutrients (e.g., vitamin A and D) that offers a range of health benefits especially at protecting against lifestyle-related diseases (Hosomi et al., 2012). Several studies have shown that fish intake could prevent heart disease risk including ischemic heart disease and arrhythmic death, reduce blood pressure, depression and others (Burger and Gochfeld, 2009; Mozaffarian et al., 2003; Raatz et al., 2013).

Despite health benefits of fish consumption, several issues emerged on the health risk associated with the marine contaminants contained in fish. A number of studies have shown the exposure of harmful pollutants including methyl mercury, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCBs), and poly-brominated biphenyl ethers (PBDEs) (Burger and Gochfeld, 2009; Domingo et al., 2007) and recently microplastics through consumption of fish which have a detrimental effect on human health if exceed the tolerance (Daniel et al., 2020; Smith et al., 2018). According to Rochman et al. (2015), microplastics can be considered as ‘cocktail of contaminants’ which are linked to a variety of chemicals pollutants either incorporated during plastics production, incineration, recycling process or through sorption of waterborne contaminants (Gallo et al., 2018; Galloway et al., 2017).

Having smaller size, microplastics are prevalence and bioavailable to a wider range of marine organisms from the smallest size of zooplankton (Cole et al., 2014; Desforges et al., 2015) to the top consumer of the ocean (Carbery et al., 2018; Fossi et al., 2014). A growing body of evidence has shown the presence of tiny size of plastics fragments in the gut, liver, gills and edible tissue of fish consumed by human, raises considerable concerns on the risk of microplastics exposure towards food safety (Abbasi et al., 2018; Barboza et al., 2020; Collard et al., 2017; Rochman et al., 2015). Laboratories evidence have shown ingested microplastics potentially causes energy depletion, fecundity (Cole et al., 2015), oxidative and neurological damage (Barboza et al., 2020; Prokic et al., 2019) and hepatic stress (Rochman et al., 2013).

The occurrence of microplastics was also reported in several marines and freshwater species in Malaysia. Ibrahim et al. (2017) demonstrate the presence of more than 4400 of threadlike shape microplastics (<1 mm) in the gastrointestinal tract (GIT) of wild and cage-cultured Asian seabass (*Later calcarifer*) sampled from Setiu Wetland, Terengganu. Nine species of commercial fish obtained from a local market in Selangor have shown to contain microplastics in approximately 38.2% of all species in their viscera and gills. Recently, Sarijan et al. (2019), discovered microplastics contamination in six species of freshwater fish sampled from Skudai River, Johor Bharu with 100% ingestion of all species.

Ingestion is considered the main pathway of microplastics uptake by fish either directly or indirectly via trophic transfer. Direct consumption occurs when plastic particles are mistakenly assumed as prey (Ory et al., 2017). Microplastics can be indirectly ingested if the fish consume contaminated prey (Lusher et al., 2016; Ryan, 2019). Therefore, the presence of microplastics is commonly observed in GIT content of fish (Karbalaei et al., 2019; Lusher et al., 2016; Sathish et al., 2020). However, few studies have reported the presence of plastics in gills suggesting microplastics can as well be taken up through the ventilation system (Abbasi et al., 2018; Su et al., 2019a).

Hence, this study aimed to quantify microplastics distribution on commercial marine fish sampled from Malaysian coastal water. The fishes were collected from two locations in Peninsular Malaysia (Fig. 1). Further, the occurrence of microplastics in GIT and gills are compared and the abundance of microplastics between locations are investigated. Chemical identification of selected microplastics is carried out using ATR-FTIR and micro-RAMAN spectroscopy. Finally, the pattern on surface morphology and variation of elemental composition of selected microplastics are observed using field emission scanning electron microscopy- energy dispersive X-ray spectroscopy (FESEM- EDX).

## 2. Materials and methods

### *2.1. Sample collection*

Sample of commercial marine fishes were trawled at Tanjung Penyabung, Mersing, Johor involving a total of 94 marine fishes from ten species. While Pantai Remis samples were purchased through local fisherman, comprised of 64 fishes from seven species (Table 1). The location of Mersing is in the south-east region of Peninsular Malaysia, which is far from the major city area. While, Pantai Remis is located in the west region where industrial, shipping, trading, and infrastructure activities are concentrated in the area. Since the fishes from Mersing were caught through trawling, the quantity of fishes was inconsistent between species. Meanwhile, samples from Pantai Remis were purchased from local fishermen. Hence, the number of fish were quite consistent except for *Alectis indica.* A short interview confirmed the fish were caught from nearest coastal water. Fish species were identified, and pictures were taken for each species for further verification. Fishes were stored in a freezer at the temperature of -20 °C until analysis. The location of Mersing is in the south-east region of Peninsular Malaysia, which is far from the major city area. While, Pantai Remis is located in the west region where industrial, shipping, trading, and infrastructure activities are concentrated in the area.

### *2.2. Sample preparation*

Fish samples were thawed at room temperature, and each individual was inspected for any deformities. Subsequent measurements of total length/cm and weight/g were taken. Fish was dissected from oesophagus to anus of fish (Lusher et al., 2016) and GIT and whole gill rakers were removed (Su et al., 2019a). GIT and gills were then weighted and placed into individual beakers containing potassium hydroxide, KOH solution (10% *w*/*v* ChemPur). To speed up the dissolution of organic matter, the volume of solution was increased to a ratio of 1:6 (organ to volume of KOH) (de Vries et al., 2020), as previous recommendation was in a ratio of 1:3 (Foekema et al., 2013). The beakers were sealed and stored in the oven at temperature of 40 °C for 72 h following digestion method from Karami et al. (2017). Such conditions could accelerate the digestion process while maintaining the integrity of potential microplastics (Jaafar et al., 2020). After being incubated for three days, the remaining materials were filtered through two stainless steel sieves (Endecotts Ltd. London) of two sizes (500 μm and 63 μm) as suggested by (Jaafar et al., 2020; Lusher and Milian, 2018). Bigger mesh size was placed at the top and if possible, another sieve with size 250 μm can be inserted into the arrangement depending on the condition of digestate. Material remaining on each sieve was rinsed with distilled water, backwashed and transferred into a clean glass petri dish. The residue on the first sieve was inspected using a microscope and possible microplastics were manually sorted out and kept in lidded glass vials. While, materials on 63 μm sieve were subjected to vacuum filtration over glass microfiber filter (1.0 μm, 47 mm GF/B filter, Whatman, USA). The filter was lightly rinsed with distilled water, transferred into a glass petri dish and was oven dried at 40 °C overnight. Overview on the digestion procedures is displayed in Fig. S1.

All glassware and apparatus used were cleaned with distilled water and rinsed with ethanol and oven dried. Unused glassware was covered with aluminium foil to avoid airborne contamination. Filter papers were

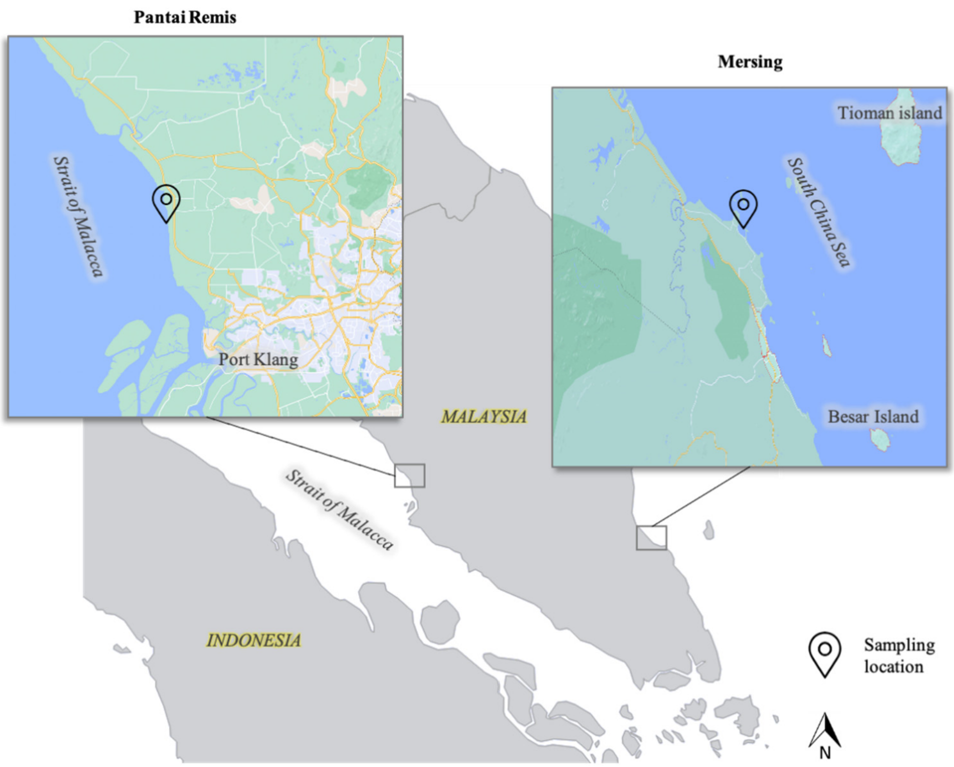


**Fig. 1.** The map of sampling stations located in Pantai Remis, Selangor, Malaysia (3°, 12”, 12.5” N, 101°, 18’, 21.7” E, DMS) and Mersing, Johor, Malaysia (3°, 13’. 24.7” N, 101°, 24’,19.7” E, DMS). Source: Google Maps (Google, nd)

inspected before use, using a digital microscope (Dino Lite, USA). To minimise contamination, GIT and gills were immediately transferred into conical flask and covered with aluminium foil. Sample blanks (at least three) were used alongside real samples prior to chemical digestion to identify potential contaminants during procedure. Particles that found on vacuum filter paper of blank sample were excluded from subsequent analysis.

## 2.3. Microplastics characterization

### 2.3.1. Visual identification

Potential plastics fragments were determined following criteria established by Lusher et al. (2017) which include an even thickness and colour across targeted materials, absent of cellular and organic matter, must be glossy and plastics fibres need to have three-dimensional bending. Microplastics (>500 μm) which were sorted and materials retained on filters (>63 μm, <500 μm) were visually inspected using a microscope and counted. Hot needle test technique was carried out to distinguish between microplastics and organic matter such as fish scale or shells (Hidalgo-Ruz et al., 2012; Lusher et al., 2017). Plastics materials were melted or curved when subjected to hot needle, while no changes were observed for non-plastics materials.

Picture of each potential microplastics was taken using a digital microscope equipped with an in house software (Dino Lite, USA) for further confirmation and details such as colour, size (length) and type were recorded. Microplastics were assigned to three particle shape categories: fragments, fibres and filament. The length of microplastics were measured at their largest cross section and categorised into four size classes: 0.063-0.1 mm, 0.1-0.5 mm, 0.5-1.0 mm and 1.0-5.0 mm following (Desforges et al., 2014). The size limit set in this study depended on

**Table 1**
General data on commercial marine fish purchased from Mersing and Pantai Remis.

| Scientific name | Common name | Location | Sample size (n) | Habitat | Average weight (g), (±SD) | Average fish length (cm), (±SD) |
|---|---|---|---|---|---|---|
| *Chirocentrus dorab* | Wolf herring | Mersing | 18 | Pelagic | 75.11 (± 37.79) | 28.43 (± 4.51) |
| *Drepane longimana* | Band sickelfish | Mersing | 14 | Demersal | 55.28 (± 44.58) | 13.83 (± 5.05) |
| *Drepane punctata* | Spotted batfish | Mersing | 3 | Demersal | 97.90 (± 44.12) | 17.33 (± 1.63) |
| *Eubleekeria jonesi* | Jones' pony fish | Mersing | 10 | Demersal | 20.42 (± 7.87) | 10.83 (± 1.68) |
| *Gazza minuta* | Slimy pony fish | Mersing | 9 | Demersal | 33.16 (± 7.43) | 14.24 (± 1.13) |
| *Gerres erythrourus* | Deep body mojarra | Mersing | 13 | Demersal | 99.42 (± 51.73) | 16.35 (± 2.77) |
| *Sardinella gibbosa* | Sardine | Mersing | 8 | Pelagic | 26.35 (± 13.96) | 12.83 (± 1.52) |
| *Triacanthus nieuhofi* | Silver tripod fish | Mersing | 5 | Demersal | 38.80 (± 14.25) | 14.16 (± 2.32) |
| *Tripodichthys blochii* | Longtail tripod fish | Mersing | 10 | Demersal | 49.57 (± 24.78) | 15.68 (± 2.79) |
| *Carangoides hedlandensis* | Bumpnosed trevally | Mersing | 4 | Pelagic | 35.10 (± 11.58) | 11.98 (± 0.93) |
| *Atule mate* | Yellowtail scad | Pantai Remis | 11 | Pelagic | 66.40 (± 12.48) | 16.99 (± 1.03) |
| *Drepane punctata* | Spotted batfish | Pantai Remis | 14 | Demersal | 77.32 (±20.93) | 13.11(± 1.18) |
| *Trachurus japonicus* | Jack mackeral | Pantai Remis | 10 | Pelagic | 72.80 (± 15.17) | 18.05 (± 1.35) |
| *Johnius borneensis* | Hammer croaker | Pantai Remis | 8 | Pelagic | 81.71(± 18.94) | 30.57 (± 1.24) |
| *Panna microdon* | Panna croaker | Pantai Remis | 9 | Demersal | 104.17 (± 30.11) | 19.39 (± 1.37) |
| *Alectis indica* | Indian threadfish | Pantai Remis | 2 | Pelagic | 118.65 (± 24.11) | 21.00 (± 0.28) |
| *Megalaspis cordyla* | Hardtail scad | Pantai Remis | 10 | Pelagic | 97.62 (± 25.23) | 21.66 (± 1.92) |
| Total | | | 158 | | | |

the minimum mesh size used during the filtration step. While, the colour category was broad and was determined by the distribution colour of microplastics in this study (e.g., black, blue, red, grey and others).

### *2.3.2. Chemical identification*

A total of 20 microplastics were selected for spectroscopy analysis to confirm and identify the type of plastic polymer. Plastics with size >500 μm were analysed using a Cary 630 FTIR spectrometer equipped with attenuated total reflection, ATR (Agilent Technologies, Mulgrave, Australia). Six microplastics were compressed against diamond crystal. For each sample, spectra were collected in transmission mode, background and sample scans were recorded using 16 co-added scans, in the range of 4000 to 600 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. Spectra were analysed using KnowItAll academic edition software (Bio Rad) and were compared with previously published spectra by Jung et al. (2018). Selected microplastics with size <500 μm retained on filter paper were labelled and analysed using confocal micro Raman imaging spectroscopy (Thermo Scientific, USA) at a wavelength of 532 nm. Collected spectra were baseline corrected to enhance the quality of spectra. Spectra were matched between recorded spectra and reference from Dong et al. (2020) and KnowItAll library database.

### *2.3.3. Surface morphology and elemental analysis*

Following chemical identification, surface morphology and elemental analysis of four selected microplastics (>500 μm) were analysed using Field Emission Scanning Electron Microscopy (FESEM) (Carl Zeiss Merlin, Compact, Germany) equipped with EDX spectrometer an energy dispersive X-ray spectroscopy system (Oxford INCA, Oxford, U.K.). Four individuals microplastics were selected and mounted on double sided adhesive carbon on aluminium stubs and coated with a very thin layer of gold (Au) using an ion sputtering instrument to obtain a better quality of SEM image. Samples were imaged at 50× - 1000× using the backscattered electron detector (BSE). By operating at 20 keV under backscatter mode, qualitative elemental composition of the surface particles was detected. Several trace metals were detected including Cd, Cr, Cu, Ni, Pb, manganese (Mn) and zinc (Zn).

### *2.3.4. Statistical analysis*

All statistical analysis was done using SPSS Statistic software (IBM, Version 25). Data were tested for normality and homogeneity using Levene's test and Shapiro-Wilk test and parametric and nonparametric were chosen accordingly. To test the significant difference between abundances of microplastics in GIT and gills, a non-parametric Mann-Whitney test was performed. The effect of microplastics shape, size and colour on abundances of microplastics in GIT and gills were determined using Kruskal Wallis test. Significant between groups were determined when probability level, $p < 0.05$.

## **3. Result and discussion**

### *3.1. Occurrence of microplastics in commercial marine fish in Malaysia*

A total of 158 fishes were examined for the presence of microplastics in GIT and gills. The most common species among the sample were *Chirocentrus dorab* (11%), *Drepane punctata* (11%), *Drepane longimana* (8%), and *Gerres erythrourus* (8%), *Atule mate* (6%), *Eubleekeria jonesi* (6%), *Tripodichthys blochii* (6%), *Trachurus japonicus* (6%), *and Megalaspis cordyla* (6%). *Other species includes Gazza minuta* (6%), *Panna microdon* (6%), *Sardinella gibbose* (5%), *Johnius borneensis* (5%), *Triacanthus nieuhofi* (3%), *Carangoides hedlandensis* (3%) and *A. indica* (1%). The size of individual fish in this present study differ across species, as commercial fishes from Mersing were caught from trawl fishing while, Pantai Remis's fish obtained for commercial purpose. According to de Vries et al. (2020) the size of fish is not related to the incident of microplastics in GIT as the process of ingestion, excretion and retention can be random. Hence, we assumed, the incident of microplastics in this study was size independent.

The overall finding indicates 86% of commercial marine fishes were vulnerable to microplastics ingestion, while 92% of the fishes were affected by microplastics through a ventilation system. The uptake of microplastics may differ based on several factors such as tropic diet, position of fish in through water column, selective feeding and others, however this study focuses only on the distribution of microplastics in commercial fishes for human consumption. The incident of microplastics obtained in this present study was significantly higher that previous literature reported in Malaysia such as in Selangor (Karbalaei et al., 2019) and Skudai river, Johor (Sarijan et al., 2019) with microplastics occurrence in only 34% and 40% of examined fishes respectively. Low incident of microplastics was also reported in fishes from other countries including 28% of fish from Indonesia, 25% from USA (Rochman et al., 2015), 11.3% of commercial fish from Saudi Arabia and only 1.68% reported in Newfoundland, Canada (Liboiron et al., 2018). However, high occurrence of microplastics was reported by Wieczorek et al. (2018) in the guts of 73% mesopelagic fishes from the Northwest Atlantic.

Variability in microplastics occurrence across study could be mainly attributed by the difference in extraction method (e.g., size limitation of microplastics, chemical digestion, filtration,) employed by each study, the location of the catching, feeding strategy and gut structure of the fish (Abbasi et al., 2018; Lusher et al., 2016). Size limitations chosen were varied across the study and primarily influenced by the size of sieve or filter used. For example, a low incident of microplastics reported by Baalkhuyur et al. (2018) was due to the large mesh size (0.2 mm) used to filter the digestate of GIT, thus discriminating against plastics items with size lower than 0.2 mm. A relatively large mesh size of 4.75 mm and 1 mm were used by Liboiron et al. (2018), explaining the low incident of microplastics in *Gadus morhua*, *Salmo salar* and *Mallotus villosus* in Atlantic. Additionally, the microplastics extraction was done without intervention of chemicals, hence could overlook any potential microplastics that are still embedded with biological tissues. Although chemical digestion was employed in the extraction procedure, Rochman et al. (2015) only quantified plastics debris with size >500 μm, avoiding the detection against microplastics with size less than that.

The exposure of microplastics and their associates in the human food chain raised considerable concern on the long-term effect of the intake of fishes captured from Malaysia waters. Until now, there is no legislation on the presence of microplastics in seafood due to inadequate exposure and hazard data hence, thus, the risk of microplastics to human health is still uncertain (Efsa, 2016; Smith et al., 2018).

### *3.2. General classification of microplastics*

A total of 1118 of microplastics were detected in commercial fish comprising of plastics fibres (80.2%), fragments (17.7%) and filaments (3.1%). Upon checking the blank sample, no microplastics were observed on the filter. Therefore, all the microplastics retained on the sample filter were included in the analysis. The high occurrence of plastics fibres found in this current study matched with previous reported incident of microplastics in fishes from other places including in Sydney harbour (Halstead et al. (2018) and the USA (Rochman et al., 2015) which comprised of 83% and 80% of fibres respectively that associated with textile fibres derived from domestics discharged. However, the abundance of plastics fibres found in this study was higher than reported in commercial fishes in Malaysia including in Selangor with only 16.3% of overall microplastics (Karbalaei et al., 2019) and in Skudai River, Johor with 20.9%. Yet, a study conducted by Ibrahim et al. (2017) found microplastics with a thread like structure were contributed to about 63.90% of overall microplastics in wild *Lates calcarifer* caught in Setiu wetlands, Terengganu.(See Fig. S2 for example of microplastics isolated from GIT and gills of examined fish).

Dominant size category of microplastics in this present study ranging from 0.1- 0.5 mm (36.3%), and 0.5-1.0 mm (31.9%) (Fig. S3). The remaining category were 1.0- 5.0 mm (27.7%) and 0.063-0.1 mm (4%). Plastics fibres were dominant in size >0.1 mm, while the majority of plastics fragments were within size of 0.063-0.1 mm. The occurrence of plastics filaments was the least with dominant size was <0.1 mm. The most prominent colour of microplastics was blue (31.9%), black (31.1%), red (19.5%), grey (12.3%), and the rest containing other colours including green, pink, yellow and purple which made up a minor fraction of microplastics abundances. Most of the plastics fibres found in this current study were black in colour, unlike fragments, the dominant colour was blue, while the majority of filaments was in red colour. Microplastics within the size of 0.5-5.0 mm appeared in black colour, however for the size category of 0.063-0.1 mm, blue colour is dominant.

According to Wieczorek et al. (2018), microplastics extracted from marine organisms can be an indicator of plastics pollution in the environment they inhabit. Hence, the classification of microplastics in terms of shape, size and colour is crucial to determine the source and impact of microplastics. For instance, plastic fibres found in an organism are associated with the clothing fibres discharged from final effluents into the ocean or originated from abandoned fishing lines or nets in the ocean (Hidalgo-Ruz et al., 2012; Khalik et al., 2018). While plastics fragments correspond to commercial plastics that are directly discarded into the ocean through anthropogenic activities (e.g., tourism, fishing, offshore installation) or as a result of poor waste management strategies of some countries (Rochman et al., 2015).

Since the size has greater influence on the impact of microplastics on the organism, reporting the size of microplastics is important to measure plastics' capacity of different size to interact with the organism which increases with the reduction of plastic's size (Lee et al., 2013; Rodríguez-Seijo and Pereira, 2017). For example, ingestion of larger microplastics can result in the injury of digestive organs or gills filaments as they pass through the organs (Ryan, 2019; Su et al., 2019a; Von Moos et al., 2012). Considering, the high tendency of smaller microplastics to interact with toxic pollutants (e.g., plasticisers, polybrominated diphenyl ether (PBDE), heavy metals, polychlorinated biphenyls (PCBs)), they have a greater impact on organism particularly at the cellular level (Prinz and Korez, 2019). Plastic with size <1 mm able to penetrate the cell barrier and be transported beyond the digestive tract or gills and causes adverse effect such as oxidative damage, fecundity and immune response on the organism (Abbasi et al., 2018; Browne et al., 2008; Collard et al., 2017; Prokic et al., 2019). Classification of plastics according to colour have been highlighted recently to determine the origin, feeding habits of organisms and potential impact of microplastics (Khalik et al., 2018; Sathish et al., 2020). For example, Sathish et al. (2020) reported a relatively high occurrence of blue plastics extracted from GIT of fishes from India's Southeast coast corresponding to the blue fishing gears used in the region. Another study found a greater incident of blue microplastics in GIT of visual predator, *Decapterus muroadsi* fish which mistakenly consumed plastics particles that resemble blue copepods prey (Ory et al., 2017). Some of coloured plastics were reported to contain toxic metals associated with inorganic colourant incorporated during plastics manufacture, potentially imposing additional risk towards environment and organisms (Massos and Turner, 2017; Nakashima et al., 2011).

### *3.3. Microplastics variation between locations*

Based on Fig. 2, the occurrence of microplastics in fishes from Pantai Remis were higher than in Mersing. About 632 pieces of plastic items were retrieved from 64 fishes from Pantai Remis and 486 pieces from 95 fishes in Mersing. Both areas showed a comparable proportion of fibres and fragments, except for filaments, which are more abundant in Mersing (Fig. S4). Majority of microplastics were within the size of 0.1-1.0 mm for both areas.

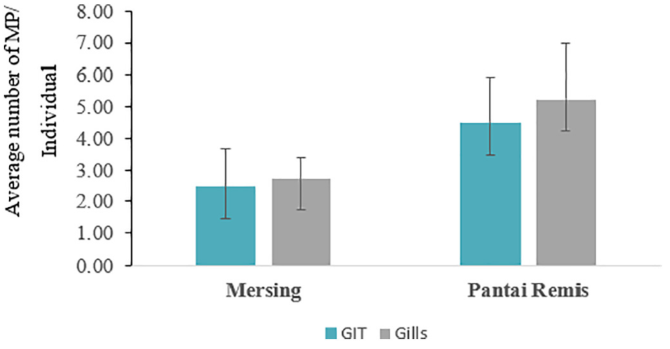


**Fig. 2.** Average number of microplastics found in GIT and gills per individual between Mersing and Pantai Remis. Error bar represent the standard deviation of average number of MP in GIT or gills across species.

The distribution of microplastics' colour was comparable with black and blue were dominant in both locations. Overall, the high occurrence of these colours were similar with the report of several studies in various location including Northwest (Wieczorek et al., 2018) and Northeast Atlantic Sea (Lusher et al., 2016; Murphy et al., 2017), North and Baltic Sea (Rummel et al., 2016), Southeast coast of India (Sathish et al., 2020), Northern Tunisia (Abidli et al., 2019), central coast of Portugal (Bessa et al., 2018), Iceland (de Vries et al., 2020) and Mediterranean Sea (Güven et al., 2017) which closely linked with the fishery activities.

High incidence of microplastics in fishes from Pantai Remis may be due to the proximity to the urban area and anthropogenic activities. The location itself is densely populated and is visited by local people and tourists during weekends or public holidays. The recreational and tourism activities increase the plastics littering pressure which is most at risk of entering nearest coastal water through wind or tidal currents. Further, Pantai Remis coastal water is connected to Sembilang River which receives disposal of effluents generated from the Jeram Sanitary Landfills (Tengku Ibrahim et al., 2017). The landfills accept about 2535 tons a day of waste and about 95% of incoming waste are derived from domestics including plastics waste (Malakahmad et al., 2017). A systematic study conducted by Tengku Ibrahim et al. (2017) found the river is contaminated by the effluent from the landfills operation. Although the author focussed on the water quality of the river without analyzing the occurrence of microplastics, evidence of the presence of plastics particles in the landfill's leachate has been reported in other countries. For example, Su et al. (2019b) reported about 8 ± 3 plastic items/L of leachate, mostly fibres shaped in Laogang landfill, in Shanghai, China. According to Fazeli et al. (2016), the growth rate of waste dumped in the landfills was estimated to increase at a rate of 3.3% yearly and projected to receive more than 1,160,000 tons of waste in 2022. Hence, it is expected that the discharges of effluent through Sembilang River may contribute to the abundance of microplastics found in fishes from Pantai Remis coastal water. Coastal fishing of seawater fish is a popular activity among visitors in Pantai Remis. Further, commercial fishing involves coastal fisheries by small-scale fisher to provide fish supply for local commodities is another main activity along the coast area. Hence, these activities can as well contribute to the loss of fishing gears or nets in coastal water which eventually enhance the burden of plastics waste in the area.

Mersing district located in the southeast of peninsular Malaysia and surrounded by 93 islands of which five islands are inhabited and others are known to be major tourist destinations, which include Sibu Island, Rawa Island, Tengah Island and others. Despite being popular among locals and foreigners, there were no proper methods of disposing of waste in these areas. According to Mersing District Council, Nor Azmi Amir Hamzah in the interview with local reporter, the waste management of the islands was dependent on the resort operators or islanders (Zaliza Musa, 2020). The methods are varied including using septic tanks, directly discharged sewage directly into the ocean or circulating reusable water using an advanced system. In addition, the sampling location is located in Tanjung Penyabong, Mersing which is surrounded by five popular beaches including Pantai Pasir Lanun, Pantai Pulau Mawar, Pantai Teluk Resang and Pantai Teluk Gorek (Fig. S5). Recreation activities such as fishing, picnicking, swimming and snorkelling are common at the beaches. The economic activities in this area includes palm oil plantation, resort and fisheries which formerly negatively affected the quality of water (Rashid et al., 2020). Hence, the source of microplastics found in fishes from Tanjung Penyabong could have originated from recreational, tourism and fishery activities in this area.

### 3.4. Microplastics variation among species

Microplastics presented in all species, however, the average number of microplastics per individual were varied between GIT and gills which ranged from 0.50 to 6.25 and 1.90 to 6.90, respectively as presented in Table 2. Species with the highest number of microplastics per individuals included *J. borneensis* (GIT: 6.25 ± 1.91, gills: 5.75 ± 3.37), *T. japonicus* (GIT: 5.20 ± 2.25, gills: 6.90 ± 2.56), and *M. cordyla* (GIT: 5.60 ± 2.07, gills: 6.80 ± 3.16). *C. hedlandensis* and *E. jonesi* were recorded to have the least number of plastics items per individuals with GIT: 0.50 ± 1.00, gills: 3.25 ± 2.36 and GIT: 0.80 ± 0.92, gills:1.90 ± 1.52, respectively. It is worth noting that *J. borneensis, T. japonicus* and *M. cordyla* are pelagic feeders that feed on floating plastics (Murphy et al., 2017). Thus, explained the high occurrence of plastics in GIT and gills of these fish which accumulation of microplastics is expected to be high. The high incident of microplastics in pelagic fish has been reported in other studies at North Sea and Baltic Sea, where microplastics were observed in 10.7% of pelagic fish, while only 3.4% of demersal fish contained plastic particles (Rummel et al., 2016). According to Azad et al. (2018), about 60% of pelagic fish (*J. borneensis*) was observed to contain microplastics in the gut compared to only 44.44% of demersal (*P. microdon*) sampled from lower Gulf of Thailand.

P. Remis = Pantai Remis.

Assessing the uptake of microplastics through ingestion, some fish species may confuse plastics particles as their food, hence can actively feed on microplastics. For example, a study conducted by Barboza et al., 2020, observed similar types of microplastics in the GIT of *Dicentrarchus labrax, Trachurus trachurus* and *Scomber colias* from North-East Atlantic. According to the author, the blue plastics resemble their usual food indicating the selective feeding of the species which enhance the uptake of microplastics through ingestion. However, there are several species that are able to differentiate against inedible prey, especially fishes with chemosensory foraging style (Roch et al., 2020). Microplastics can still be taken up by these fish through trophic transfer or accidentally consumed during feeding or drinking (Ryan, 2019). Considering, more fishes are unintentionally ingesting microplastics, it can be concluded that the occurrence of microplastics in GIT are largely influenced by the level of microplastics in water (Giani et al., 2019; Roch et al., 2020).

### 3.5. Microplastics load in GIT and gill of fish

Of the 1118 microplastics found in 158 examined fish, 47% were detected in GIT, while 53% microplastics in gills. Mann-Whitney test showed the occurrence of microplastics in GIT was not statistically significant ($p > 0.05$). The occurrence of microplastics in gills were slightly higher than found in GIT (Fig. 2). The distribution of microplastics shape between GIT and gills was not statistically significant (Kruskal-Wallis test, $p > 0.05$). The percentage of plastics shape, colour and size between GIT and gills are illustrated in Fig. 3. There was statistically significant

**Table 2**
Number of fishes contained microplastics, average number of microplastic per ndividual range of MP found in GIT.

| Species | Sample size | Location | No of fish with MP, (%) | | No. of MP per fish, ±SD, (Range number of MP) | |
|---|---|---|---|---|---|---|
| | | | GIT | Gill | GIT | GILL |
| *C. dorab* | 18 | Mersing | 12 (67) | 17 (95) | 1.89 ± 1.88 (0-6) | 2.78 ± 1.52 (0-5) |
| *D. longimana* | 14 | Mersing | 13 (93) | 14 (100) | 3.43 ± 1.95 (0-7) | 3.29 ± 2.09 (0-8) |
| *D. punctata* | 3 | Mersing | 3 (100) | 2 (67) | 3.67 ± 1.15 (3-5) | 2.00 ± 2.0 (0-4) |
| *E. jonesi* | 10 | Mersing | 5 (50) | 8 (80) | 0.80 ± 0.92 (0-2) | 1.90 ± 1.52 (0-4) |
| *G. minuta* | 9 | Mersing | 9 (100) | 9 (100) | 3.78 ± 2.68 (2−10) | 3.67 ± 1.32 (2-6) |
| *G. erythrourus* | 13 | Mersing | 12 (92) | 12 (92) | 3.23 ± 2.01 (0-7) | 3.15 ± 2.38 (0-6) |
| *S. gibbosa* | 8 | Mersing | 6 (75) | 6 (75) | 2.00 ± 1.51 (0-4) | 2.13 ± 1.73 (0-6) |
| *T. nieuhofi* | 5 | Mersing | 5 (100) | 4 (80) | 3.60 ± 1.82 (2-6) | 2.20 ± 1.79 (0-4) |
| *T. blochii* | 10 | Mersing | 7 (70) | 8 (80) | 1.45 ± 1.17 (0-4) | 2.10 ± 1.85 (0-5) |
| *C. hedlandensis* | 4 | Mersing | 1 (25) | 3 (75) | 0.50 ± 1.00 (0-2) | 3.25 ± 2.36 (0-5) |
| *A. mate* | 11 | P. Remis | 10 (91) | 11 (100) | 2.55 ± 1.86 (0-7) | 3.73 ± 2.65 (1-8) |
| *D. punctata* | 14 | P. Remis | 15 (100) | 13 (87) | 3.43 ± 1.79 (2-9) | 3.71 ± 3.50 (0−12) |
| *T. japonicus* | 10 | P. Remis | 10 (100) | 10 (100) | 5.20 ± 2.25 (2-9) | 6.90 ± 2.56 (4-11) |
| *J. borneensis* | 8 | P. Remis | 8 (100) | 8 (100) | 6.25 ± 1.91 (4-9) | 5.75 ± 3.37 (1−12) |
| *P. microdon* | 9 | P. Remis | 9 (100) | 9 (100) | 5.44 ± 1.32 (4-8) | 6.44 ± 2.55 (3−11) |
| *A. indica* | 2 | P. Remis | 2 (100) | 2 (100) | 4.50 ± 0.71 (4-5) | 3.00 ± 1.41 (2-4) |
| *M. cordyla* | 10 | P. Remis | 10 (100) | 10 (100) | 5.60 ± 2.07 (4-10) | 6.80 ± 3.16 (3-11) |
| Total | 65 | | 64 | 63 | | |

correlation between microplastics size across GIT and gills (Kruskal Wallis test, $p < 0.05$). Kruskal-Wallis test showed no statistically significant difference between the distribution of colour of microplastics between GIT and gills ($p > 0.05$) Hence, reflect the abundance of microplastics' colour in the both coastal water in Pantai Remis and Mersing.

Ingested microplastics can be regurgitated, retained or egested depending on the size of marine animals and microplastics (Welden and Cowie, 2016). Several studies found plastics particles on marine animals' faeces, suggesting microplastics can as well be excreted (Ryan, 2019). The high incidence of microplastics in gills in this current study, indicates, the uptake of microplastics through ventilation is considered an important pathway of microplastics in fish (Su et al., 2019a). Microplastics clogged within gills filaments may result in several adverse effects, including physical injury on the gill filaments, and reduce respiratory efficiency, which later causes hypoxia and can be fatal (Barboza et al., 2020). Several laboratory studies have shown microplastics' capabilities through ventilation to accumulate and disperse chemical pollutants similar to ingested microplastics (Barboza et al., 2018; Zhu et al., 2020). Translocation of microplastics via gills is possible and is believed to be adsorbed by the filaments surface then endocytosed into the cell and enter the blood circulatory system (Zhu et al., 2020).

### 3.6. General chemical confirmation of microplastics

Following visual analysis, a spectroscopic analysis was performed to determine the chemical composition of plastics polymer (Jung et al., 2018; Lusher et al., 2017; Rummel et al., 2016) and the degree of plastics degradation (Chamas et al., 2020). A total of 20 microplastics were selected and subjected to spectroscopy. Plastics with size larger than >500 μm were tested using ATR FTIR, while the smaller size of microplastics (<500 μm) were analysed using Raman microscopy. Initial comparison of plastic polymers was carried out for chemical assignment by comparing the spectrum with spectral libraries Bio-Rad and SpectraBase and reaffirming by referring to a published report's spectra. All selected items were confirmed to be plastics. The identified polymers were polyamide (PA), polyethylene terephthalates (PET), polypropylene (PP), polyethene (PE), polystyrene (PS), acrylonitrile butadiene styrene (ABS) and paint particles (acrylic latex and phenolic resins). Due to the small samples of plastics subjected to chemical analysis, comparing the occurrence of plastics items based on the type of polymers across fish cannot be determined. List of microplastics of confirmed chemical assignments are presented in Table S1.

### 3.7. Fourier-transform infrared spectroscopy analysis of microplastics

Plastic debris exposed to the environment may experience surface erosion and cracking, which later led to mass loss through the fragmentation process in the presence of synergic action of heat and light (UV exposure). The subsequent process involved mechanical abrasion or degradation by microorganism (Hakkarainen and Albertsson, 2004). When subjected to an infrared spectrometer, the spectra of plastics fragments especially PP and PE often exhibit addition or increase the intensity of carbonyl absorption band (C=O) located around 1700 $\text{cm}^{-1}$, at 1027 $\text{cm}^{-1}$ (C—O) and 874 $\text{cm}^{-1}$ and 911 $\text{cm}^{-1}$ (C=C) which associated with weathering process of plastics (Ding et al., 2019; Dong et al., 2020; Fotopoulou and Karapanagioti, 2006). In this present study, additional

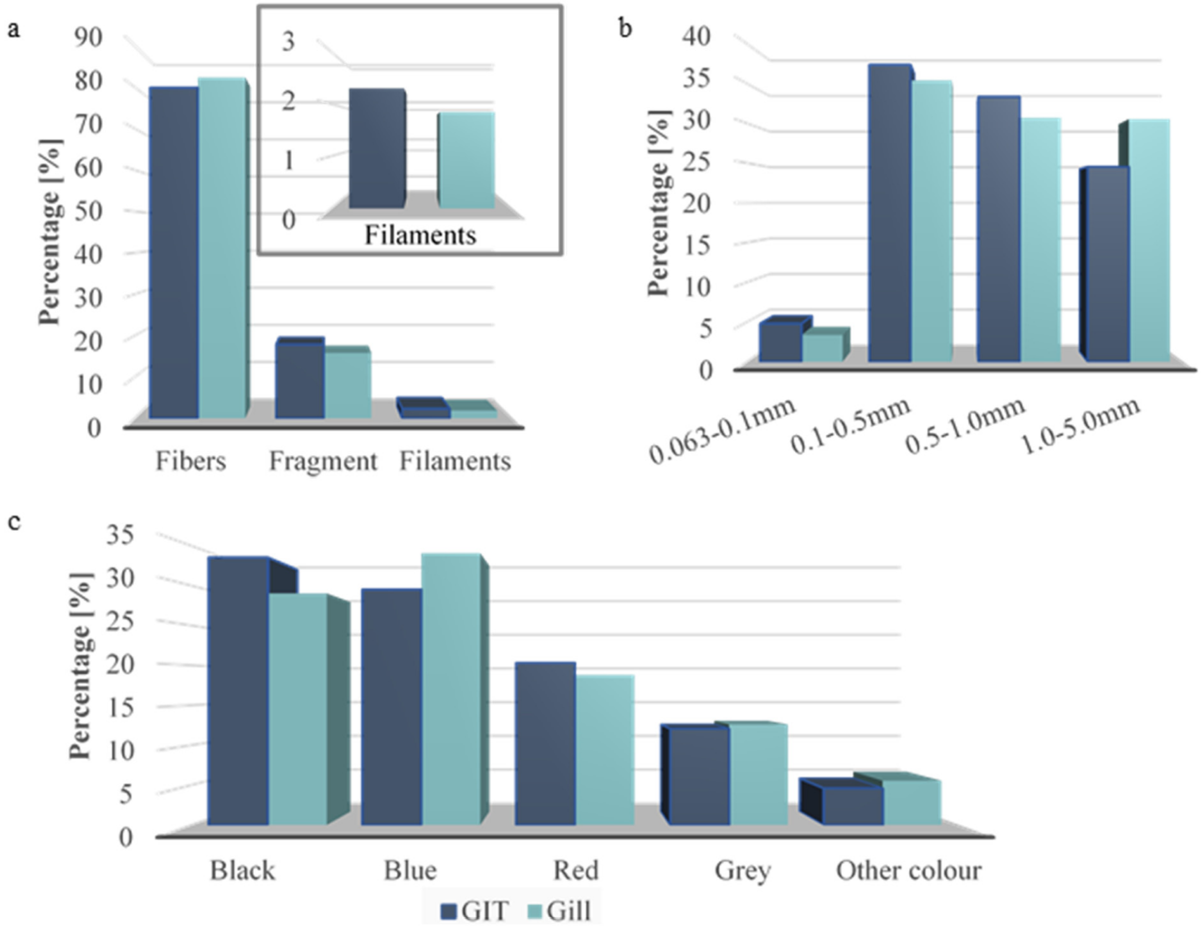
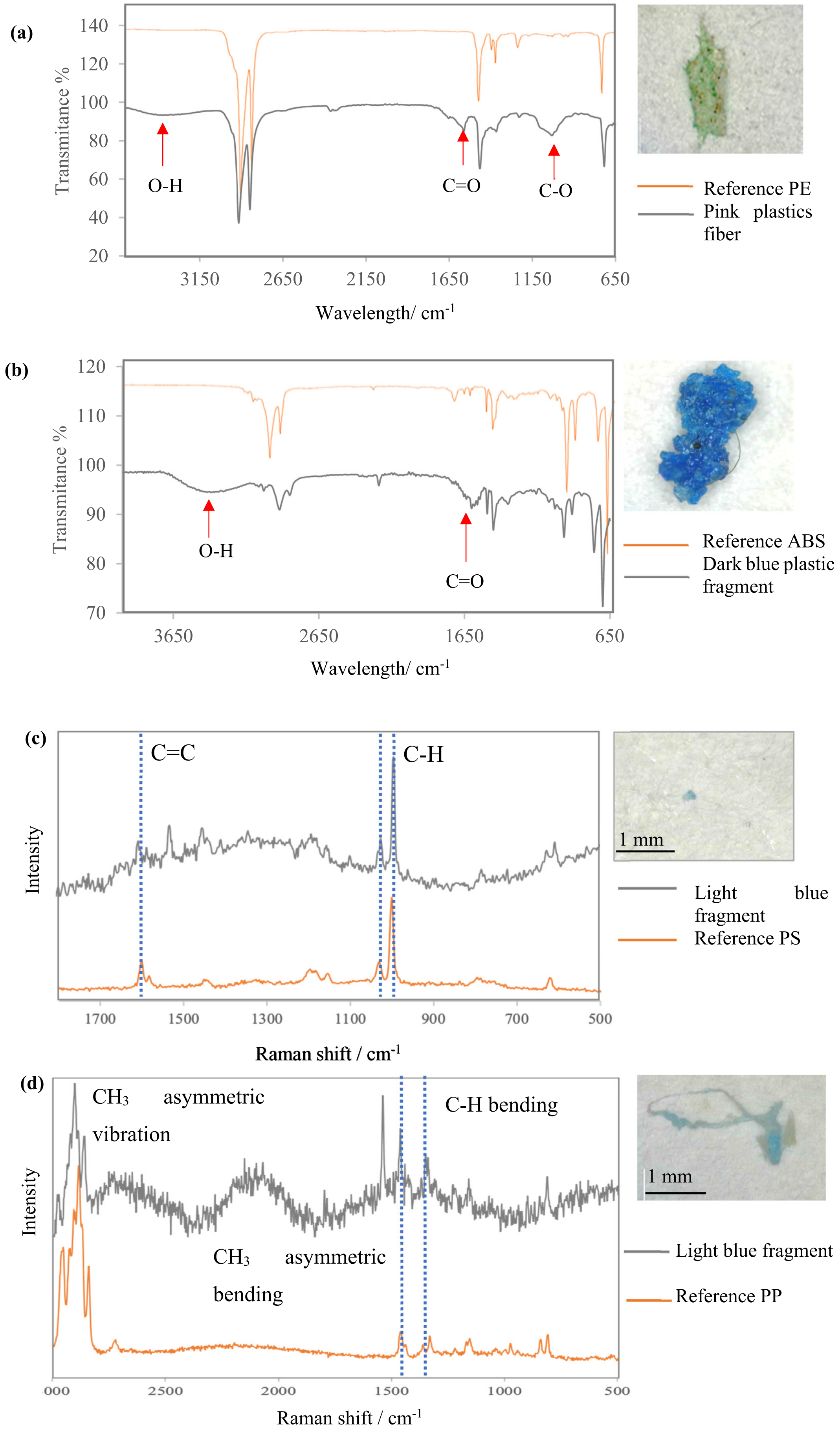


**Fig. 3.** Distribution of microplastics between GIT and gills across different (a) shape (Kruskal Wallis, $p > 0.05$), (b) colour ($p > 0.05$) and (c) size ($p < 0.05$).


(a)
Transmittance %
140
120
100
80
60
40
20
3150
2650
2150
1650
1150
650
Wavelength/ cm-1
O-H
C=O
C-O
Reference PE
Pink plastics fiber
(b)
Transmittance %
120
110
100
90
80
70
3650
2650
1650
650
Wavelength/ cm-1
O-H
C=O
Reference ABS
Dark blue plastic fragment
(c)
Intensity
C=C
C-H
1700
1500
1300
1100
900
700
500
Raman shift / cm-1
1 mm
Light blue fragment
Reference PS
(d)
Intensity
CH3 asymmetric vibration
C-H bending
CH3 asymmetric bending
000
2500
2000
1500
1000
500
Raman shift / cm-1
1 mm
Light blue fragment
Reference PP

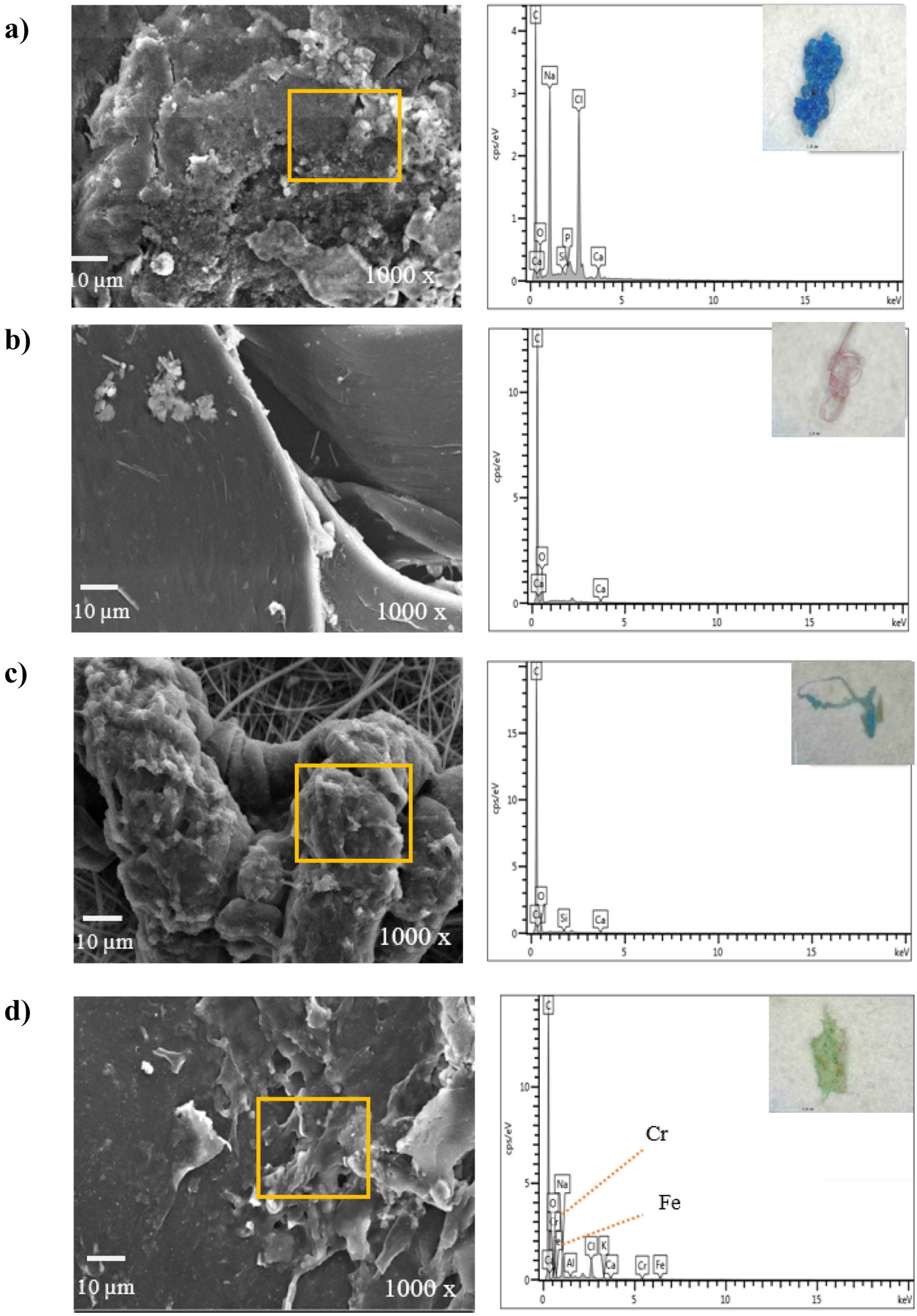


**Fig. 5.** SEM images showing morphologies of selected sites accompanied by spectrum of EDX of (a) ABS, (b) and (c) PP, and (d) PE. Strong peak intensity corresponding to carbon was observed in all spectra of microplastics indicative of plastic polymers. Cr and Fe were observed on spectrum (c) indicating interaction of microplastics and heavy metals.

**Fig. 4.** Examples of infrared and Raman spectra representative of microplastics found in the GIT and gills of fish (grey line) and corresponding reference spectra (orange line). Red arrow showing the additional bands relative to reference spectra. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adsorption bands were presence especially on PP and ABS polymer relative to the spectrum of reference polymer which corresponds to a functional group including hydroxyl or hydroperoxide, O—H (3400 cm$^{-1}$), carbonyl, C═O (1595 cm$^{-1}$) and ether, C-O-C (1045 cm$^{-1}$) (Fig. 4) (Table S2).

### 3.8. Raman spectroscopy analysis of microplastics

Raman micro spectroscopy provides a sophisticated approach at determining the chemical composition of sub-micron to micro-sized plastics particles, showing better identification of non-polar and symmetric bonds (Dong et al., 2020; Lenz et al., 2015). The detection of possible plastics extracted from various environmental and field sample using Raman spectrometer has been widely reported by numerous studies including sediment, wastage sludge (Lares et al., 2019), drinking water (Kniggendorf et al., 2019) and biota (Karbalaei et al., 2019). However, challenges of using this spectroscopic analysis arise due to the multicomponent of plastics (e.g., the presence of dye, plastics additives, filler, organic residual), UV degradation and fluorescence (Ribeiro-Claro et al., 2017).

Several microplastics appeared to have similar physical characteristics (e.g., shape, colour), however, the spectroscopic analysis showed two distinct spectrum profiles belonging to the different polymers. For instances, identical blue fragments exhibited a spectrum of PS with the characteristic peaks at 1640 cm$^{-1}$ (C═C bond stretching), and 998 cm$^{-1}$(C—H out of plane vibration), while other fragment showed strong bands at 2950 cm$^{-1}$($CH_3$ asymmetric vibration), 1460 cm$^{-1}$($CH_3$ asymmetric bending), and 1360 cm$^{-1}$ (C—H bending), representing PP polymer (Fig. 4).

### 3.9. Surface morphology and elemental analysis using FESEM-EDX

Following infrared and Raman spectroscopy, the surface morphologies of four selected microplastics were observed using FESEM combined with energy dispersive X-ray spectroscopy (EDX). According to Wang et al. (2017b), the method could rapidly differentiate plastics items from other inorganic materials such as fish scales, shells and ceramic flakes with high accuracy and minimal identification error. Further, FESEM images revealed surface characteristics of microplastics as a result of environmental exposure. Similar techniques have been used to detect contaminants, especially heavy metals on microplastic's surfaces (Karbalaei et al., 2019; Wang et al., 2017a).

Fig. 5 showed FESEM images of selected microplastics. Rough surfaces and cracks were observed which were linked to the weathering and mechanical erosion, consistent with other studies on microplastics retrieved from biological and environmental samples (Ding et al., 2019; Wang et al., 2017a). Formation of uneven and rough surfaces was common in aged microplastics, causing an increase in the surface area, which facilitated the interaction between plastics and marine pollutants, organic matter or microorganism (Fotopoulou and Karapanagioti,

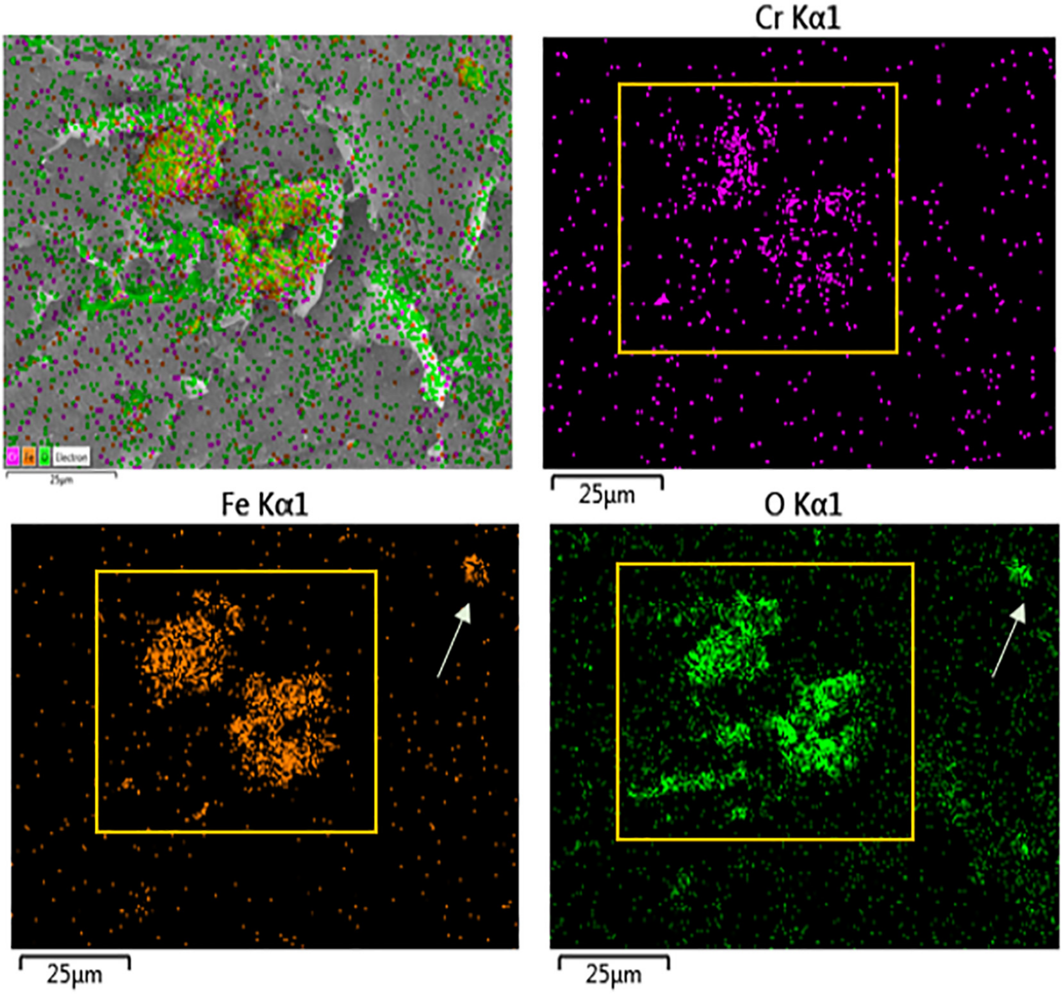


**Fig. 6.** Images of elemental mapping focusing on elements of Cr, Fe and O which concentrated at similar sites (area covered by yellow rectangle). The arrows showed the similar distribution if Fe and O which indicates the presence of iron compound. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2012). The images displayed variation on the surface texture, indicating the different degree of environmental exposure. Uniform scratching and shallow grooves were observed on PP's surfaces (Fig. 5b) illustrated mechanical erosion on the surface which developed due to the sedimentological process at which microplastics were dragged along the surface of a stationary object (Cooper and Corcoran, 2010). While smooth and blunt edges formed on the surface of blue coloured PP displayed in Fig. 5c, corresponding to the microplastics' longer residence time. The same image showed bumpy texture with a cluster of spherical pits accompanied by a deep well.

Combination of FESEM-EDX is a useful elemental analysis that allows subsequent conformation of plastics items. Consistent to spectroscopic analysis of ABS, PP and PE, elemental analysis of these microplastics exhibited high intensity of carbon peak, which affirmed the polymers' chemical composition. The EDX spectrum showed the presence of elements including calcium (Ca), sodium (Na), chlorine (Cl), and oxygen (O) which corresponded to the deposition of the salt compound. Heavy metals, namely chromium (Cr) and iron (Fe) were related to metals' adherence.

EDX spectrum of green coloured PE fragment showed heavy metals namely chromium (Cr (II)) and iron (Fe (III)) on a different part of the plastics' surface. Elemental mapping of microplastics was carried out, revealing distribution of Fe on the plastics surface was consistent with O which suggested the presence of iron compound at several parts of PE surfaces as displayed in Fig. 6. The presence of iron compound can be derived from various sources, including river runoff, resuspension of marine sediment, aeolian deposition, hydrothermal activities (Wells, 1988) or biogenic iron oxides (Emerson, 2016).

The distribution of Cr was concentrated at the similar site as Fe and O confirmed the presence of a reactive site on microplastics' surface. The presence of reactive sites are associated with the surface modification of plastics primarily due to the weathering and ageing process which provides charged sites on the surface (Ashton et al., 2010). Besides, roughness and cracking of the surface facilitate the attachment of organic matter (Godoy et al., 2019; Turner and Holmes, 2015) and promote the formation of biofilm (Richard et al., 2019; Rummel et al., 2017). Biofilm on the surface of microplastics could reduce the hydrophobicity of plastic polymers by increasing the surface oxygen-related functional group such as C—O and C═O, hence providing suitable sites for adherence of metal ions (Tu et al., 2020).

Interaction of microplastics and heavy metals have been reported by Nakashima et al. (2011) on the presence of Pb and Cr in PE plastics debris sampled from Gato Islands, Japan which are associated with the colouring pigments added during the manufacture of plastics products. It is important to note that heavy metals are widely used during plastics production either incorporated as chemical additives (e.g., flame retardants, inorganic pigments) or catalysts. Hence, the presence of certain metals (e.g., Cd, Pb, Zn and Cr) on aged or beach microplastics were extensively reported (Ashton et al., 2010; Noik et al., 2015). Due to the diversity of physicochemical properties, microplastics can interact with heavy metals from the surrounding through adsorption mechanisms. Rochman et al. (2014) have demonstrated PET, HDPE, LDPE, PP and PVC's potential to accumulate nine metals during 12- month exposure in San Diego, USA and suggested microplastics could adhere high concentration of metals than natural sediment. Brennecke et al. (2016) also reported a similar observation on the accumulation of Cu and Zn on PVC and PS, which were 800 times higher than surrounding seawater.

## 4. Conclusion

Overall, this study serves to highlight the widespread distributions of microplastics in commercial marine fishes from Mersing and Pantai Remis. Further, the high incident of microplastics in fishes from Pantai Remis reflected the plastics waste level in coastal water associated with anthropogenic activities. This study's data can be used as baseline information on the status of microplastics pollution in Mersing and Pantai Remis. These results indicate the potential of microplastics exposure in marine organisms that can induce adverse effects such as energy depletion, fecundity, oxidative and neurological damage, suffocation, starvation and ultimately lead to mortality among marine animals which can reduce marine resources. This study found plastic particles in the gills suggesting another potential organ for microplastics uptake. Therefore, reporting the abundance of plastics in both GIT and gills should be employed for reliable monitoring of microplastics contamination in fish. Additionally, larger microplastics were more concentrated in gills suggesting they were less likely to be flushed back into the seawater than small microplastics. Smaller size plastics were dominant in GIT indicates microplastics could be actively uptake by fishes by assuming plastics as prey or through trophic transfer. This study observed variation on microplastics' surface characteristics attributed by the type of degradation and microplastics residence time. The consumption of fish raised concern on the long term impact of microplastics and their associated contaminants on human health. Although GIT and gills are removed before cooking, the possible transfer of smaller-sized plastics particles and harmful contaminants into edible tissue potentially exposes humans to microplastics. Therefore, microplastics in GIT and gills of commercial fish potentially pose a threat to human food safety.

## CRediT authorship contribution statement

**Azwan Mat Lazim:** Main conceptualization, Methodology, Supervision, Reviewing and editing the manuscript.

**Hazwani Jaafar:** Data curation, Carried out the research, Writing- Original draft preparation.

**Ahmad Azfaralariff:** Discussion, Reviewing, Correction and editing the manuscript.

**Syafiq Musa**: Supervison, Consultation on investigation of microplastics in fish.

**Abdul Hafidz Yusoff**: Consultation on Human Risk asessment.

**Mazlan Mohamed:**: Conceptualization and methodology.

## Declaration of competing interest

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

We would like to draw the attention of the Editor to the following publications of one or more of us that refer to aspects of the manuscript presently being submitted.

We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149457.

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