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Quantification and Characterisation of Microplastics in Fish and Surface Water at Melayu River, Johor

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Abstract. Microplastics are plastic particles (< 5 mm) found in the environment that can be ingested by animals and transferred up in the trophic level. The study was conducted through sample collection, digestion of gastrointestinal (GI) tracts of fish, density separation using NaCl, filtration, microscopy, and ATR-FTIR Spectroscopy. The amount of ingested microplastics by Melayu River fish samples was: Gray Eel-Catfish (3.92 ± 4.17 particles/individual) > Sagor Catfish (2.00 ± 1.41 particles/individual) > Spotted Sicklefish (2.00 ± 0.00 particles/individual). The trend of microplastics by month in water samples was Mar-20 (2.89 ± 1.36 particles/L) > Feb-20 (1.33 ± 1.00 particle/L) and Jan-20 (1.00 ± 0.87 particle/L). Microplastics were mostly in the class size $0 \mu\text{m} - 0.50 \mu\text{m}$. In the fish samples, fibres were found to be dominant. In water samples, films were dominant. Ingestion of microplastics by colour was ranked as blue > black > red > yellow in fish samples whereas microplastics' colour in water samples was ranked as blue > red > black > translucent > green. Therefore, it is concluded that the abundance of blue microplastics in fish samples was due to the common blue plastics used by the locals. The identified microplastics were of polyethylene terephthalate (PET) and polyethene (PE) origins.

1. Introduction

Global plastic production increased from 359 million to 368 million from 2018 to 2019 wherein 2019, plastic production is divided into packaging (39.6 %), construction materials (20.4 %), automotive industries (9.6 %), electronic and electrical appliances (6.2 %), household, leisure and sports items (3.4 %) and 16.7 % in items in various industries (e.g., medical) (PlasticsEurope, 2020). Plastic polymers are often hard to biodegrade but if they do, they require the help of organisms that have degradation ability such as mealworms (Horton et al., 2017).

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Microplastics are plastic particles (<5 mm) that can travel long-distance by the circulation of the water or sea surface due to their buoyant properties (Density between $0.917-1.61 \text{ g/cm}^3$) and prevalent nature (Campbell et al., 2017; Thevenon & Carroll, 2015; Wu et al., 2019). Plastics can be broken down into microplastics through exposure to UV radiation which causes the plastics to become brittle.



Microplastics are divided into primary microplastics which are released readily into the environment as small particles (e.g., microbeads in facial cleanser) and secondary microplastics that include fragmentation of larger plastics (e.g., packaging) (Weis, 2020; Wu et al., 2019). Microplastics can enter the aquatic environment through fishing activities, tourist ships, toiletries, and the washing of synthetic textiles (Karbalaei et al., 2018). Surface water is reported to have a higher amount of polystyrene (PS), polypropylene (PP), and polyethylene (PE) (Wu et al., 2019).

Freshwater microplastics have become increasingly studied (Ben et al., 2021; Campbell et al., 2017). These microplastics originate from land-based sources which are dependent on the level of urbanization, plastic disposal behaviour, and common plastic usage (Ben et al., 2021). Microplastics can become a vector to harmful substances such as heavy metals and persistent organic pollutants (POPs) present in the environment and can also leach out additives that were added during the plastic manufacturing process (Wu et al., 2019). Exposure to both microplastics and chemical contaminants is said to increase toxicity levels in several organisms (Ha & Yeo, 2018).

Microplastics tend to be ingested by aquatic animals, typically by fish as microplastics can look similar to their natural prey [10,11]. The amount of microplastics that enter freshwater organisms depends on their feeding behaviour. Microphagous animal is likely to accidentally ingest microplastics along with their natural food [12]. Benthic organisms will mostly encounter high-density microplastics on the bottom sediment [6]. For fish, ingestion of microplastics will obstruct and damage their digestive tracts and can cause them to starve to death [13]. Microplastics can travel up the trophic level via the prey-predator relationship [14] and will eventually end up in the human digestive system.

The findings in this study determined the amount of ingested microplastics in common fish found in the Melayu River in Malaysia as well as the microplastic abundance in its surface water. Additionally, the findings helped to highlight safety in food consumption and the consequences of water pollution. Humans consumed up to 5 g of plastic every week due to incidental plastic intake when consuming food and drink [15]. According to several studies, fibres were most dominant in freshwater fish as fibres' shapes are flexible and easier to be ingested [16,17]. The colour of microplastics ingested by fish differs by species. Some of the species rely on visual to consume food which can be affected by the level of illumination in the aquatic environment whereas other relies on specialized chemical sense regulators to detect potential food [17]. Although this study focused only on the gastrointestinal (GI) tracts of fish, other parts of the fish such as gills can also accumulate microplastics [18]. In Johor, Malaysia, there is still a lack of studies on microplastics, particularly in surface water. In Skudai and Tebrau rivers, the mean microplastics in sediment were 200 ± 80 particles/kg and 680 ± 140 particles/kg respectively. However, sediment acts as a sink for microplastics and therefore contained a higher amount of microplastics [19,20]. In addition, both rivers were located in a highly-populated area as opposed to the Melayu River.

2. Methodology

This section detailed the methodology for this study. Methodology used in this study included site selection, collection and dissection of fish, collection and filtration of water samples, verification of microplastics and analysis of data.

2.1. Site selection

Melayu River ($1^{\circ}27'20.8''\text{N}$, $103^{\circ}41'31.3''\text{E}$) is located in Johor Bahru, Johor, Malaysia. The river starts from the residential area in Nusajaya (Horizon Hills) and flows southeast towards Kampung Sungai Melayu. The mouth of the river is located at Kampung Sungai Melayu and the river water discharges towards the Straits of Johor. A microplastic study was conducted as the Melayu River is susceptible to various pollutants due to its vicinity to residential establishments. Additionally, the river is the main source of income for local fishermen residing in Kampung Sungai Melayu. The village itself is surrounded by a vast mangrove forest which is a stopover for migratory birds. The river was reserved

for eco-tourism activities. Among the activities include a tour along the river, crab catching, bird watching, fishing, and handicrafts. Figure 1 and Table 1 show the sampling locations.

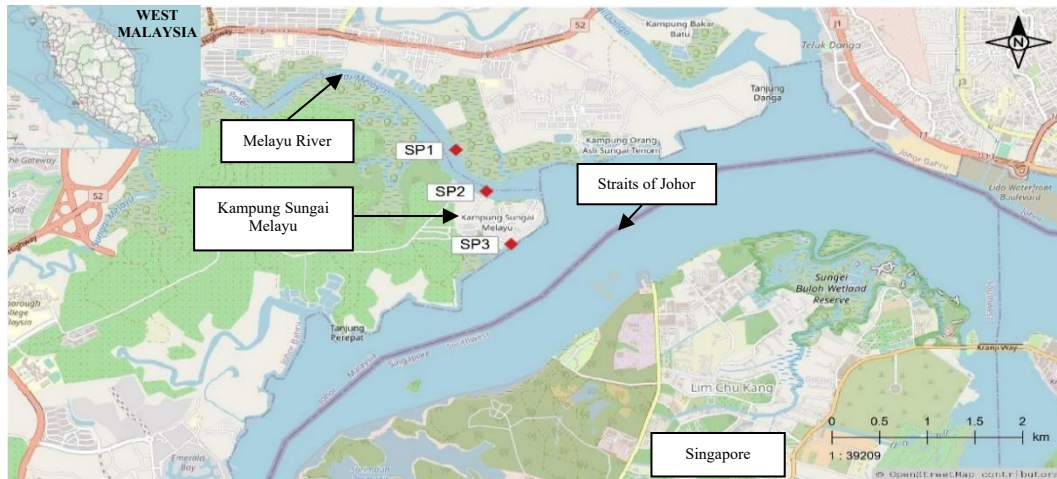


Figure 1. Sampling locations along Melayu River.

Table 1. Coordinates of the sampling locations.

Label	Location	Significance	Latitude	Longitude
SP1	Port Memancing Kampung Sungai Danga	Upstream	1° 27' 35.9" N	103° 41' 20.0" E
SP2	Restoran Sri Pantai Asam Pedas Kampung Sungai Melayu	Middle	1° 27' 19.3" N	103° 41' 31.0" E
SP3	Kampung Sungai Melayu	Downstream	1° 26' 58.8" N	103° 41' 39.3" E

2.2. Collection and dissection of fish

Spotted Sicklefish (*Drepane punctata*), Sagor Catfish (*Hexanematichthys sagor*), and Gray Eel-Catfish (*Plotosus canius*) are some fish species that inhabit the study location. They are both eaten and sold at the local wet market. Sagor Catfish and Gray Eel-Catfish are amphidromous [21] which means species that migrate from freshwater to sea and vice-versa whereas Spotted Sicklefish can be found in shallow coastal water [22]. Additionally, Spotted Sicklefish is described as amphidromous in the FishBase database.

The fish were wild-captured on 6th January, 20th February, and 9th March 2020. They were kept in a basin with some in-situ water and transported back to the Environmental Laboratory of Universiti Teknologi Malaysia (UTM). The fish body weight (g) and total length (from snout to tail) (cm) were recorded before carefully removing their GI tracts using a knife and kitchen scissors. The GI tract (oesophagus, stomach, and intestine) was isolated carefully from other tissues, weighted, stored in a petri dish, and refrigerated until further study.

The method of extraction of the GI tract of fish followed [23] with modifications. 10% potassium hydroxide (KOH) was used for the digestion of GI tracts. To obtain 10% KOH, 100 g KOH pellets were weighed and diluted in a beaker using distilled water up to the 1000 mL mark. GI tracts were put into separate pre-labelled 250 mL conical flasks and added with 10 % KOH up to 3 times the volume of GI tracts. The conical flasks were covered with aluminium foil to avoid airborne contamination. The GI tracts were left to digest for 72 hours at room temperature to prevent heat damage to microplastics. After digestion, NaCl solution (Density 1.2 g/cm³) was used to float microplastics in the solution. The conical flasks were gently swirled and left to settle overnight. Then, the supernatant formed was filtered through

a vacuum pump using filter papers with 22 μm pore size (Grade 541, \O 47 mm, Whatman®). The moist filter paper was oven-dried at 40 °C until a constant weight was achieved.

2.3. Collection and filtration of water samples

3 of 1 L of surface water samples were collected at each sampling location on the same day as fish specimen collection. Until further steps, the water samples were stored in a dark cold room at 4°C [24] in the Environmental Laboratory of Universiti Teknologi Malaysia (UTM) to preserve the water samples and the fish samples were stored in a refrigerator. The water samples were filtered through a vacuum pump using filter papers with 22 μm pore size (Grade 541, \O 47 mm, Whatman®). The moist filter paper was oven-dried at 40 °C until a constant weight was achieved [24].

2.4. Visual inspection of microplastics

Microplastics were identified using a stereomicroscope (Model: HSZ-600) after the filtration process. The magnifications were set from 10x - 40x. Particles of organic compounds and tiny grains of sand were carefully removed. Particles that were untearable by tweezers were identified as potential microplastics and were measured using *i-solution* Premium software. 'Hot needle' test was also used to confirm suspected microplastics [4]. The microplastics obtained were classified based on their shape and colour. The colour definitions were black (including grey), red (including pink), yellow (including orange), blue (including green), white and translucent. Microplastics are divided into 5 shapes; film, fibre, fragment, bead, and foam. As microplastics are plastic particles of size 5 mm and less, i.e., plastic particles larger than 5 mm have been omitted from this study.

2.5. Verification of microplastics

ATR-FTIR analysis was conducted to verify and characterize microplastics in the samples as it reduces false-positive data by using chemical confirmation as opposed to using only a microscope [25]. The microplastics particles were transferred into a clean petri dish with new filter paper and analysed between wavelengths of 650 - 4000 cm^{-1} [26].

2.6. Analysis of data

The abundance of microplastics in fish samples (Mean \pm SD and percentage) in terms of quantity, class size, shape, and colour were plotted using OriginPro 2021. Similarly, the result for microplastic abundance in water samples was plotted using the software. The functional groups of microplastics were determined by comparing the ATR-FTIR results with past studies [27].

3. Results and discussion

This section detailed the results obtained from this study and also the discussion on the acquired data.

3.1. Physical parameters of fish species

Microplastics are either ingested by fish and other animals and might sink to the bottom and be wedged between the sediments. The total amount of microplastics ingested by Spotted Sickfish (*Drepane punctata*) were 2 particles whereas Sagor Catfish (*Hexanemataichthys sagor*) and Gray Eel-Catfish (*Plotosus canius*) ingested a total amount of 4 and 47 particles respectively. The abundance of microplastics (Mean \pm SD) in each fish species were 2.00 \pm 0.00 particles/individual, 2.00 \pm 1.41 particles/individual, and 3.92 \pm 4.17 particles/individual respectively. This was because only a small number of Spotted Sickfish and Sagor Catfish were able to be captured during the three (3) months of sampling whereas Gray Eel-Catfish can be found abundant in the area. The amount of ingested microplastics ranged from 0 - 9 particles/individual. Sagor Catfish ingested more microplastics than Spotted Sickfish due to their larger GI tracts.

The total microplastics in sampling 1 (Jan-20), 2 (Feb-20), and 3 (Mar-20) were 9, 12 and 26 particles respectively and the range of microplastics in water samples was 0 - 6 particles/L. The abundance of microplastics (Mean \pm SD) in the water samples were 1.00 \pm 0.87 particle/L, 1.33 \pm 1.00 particle/L and

2.89 ± 1.36 particles/L respectively. Water samples collected in March 2020 has the highest number of microplastics. According to the fishermen in the area, the mating season of mussels occurred during March. Thus, the burying behaviour of the mussels may have contributed to the turbid water during March 2020 and helped to dislodge microplastics from sediment. Microplastics can resuspend on the surface water due to disturbances of sediment by benthic organisms' activities [4]. The number of microplastics in fish specimens was higher than in the water samples. The presence of biofilm on the microplastics' surfaces may have sent chemical signals to trigger the feeding response in fish [14].

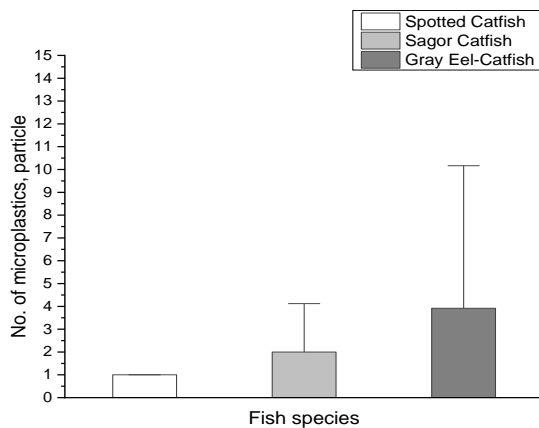


Figure 2. Number of microplastics ingested by fish specimens.

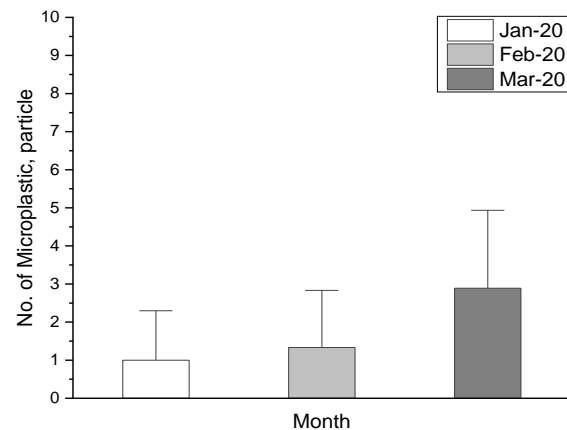


Figure 3. Number of microplastics in water samples.

3.2. Size of microplastics

The microplastics identified ranged from $0.22 \mu\text{m}$ - $3220 \mu\text{m}$. The microplastics that were of size $0 \mu\text{m}$ - $0.50 \mu\text{m}$ were recorded as the highest (Spotted Sicklefis, $n=0$; Sagor Catfish, $n=0$; Gray Eel-Catfish, $n=23$) at 0 %, 0 % and 100 % respectively (refer Figure 4). Smaller particles are easier to be accidentally ingested than bigger particles [14]. Meanwhile, microplastics that were of size $1.51 \mu\text{m}$ - $2.00 \mu\text{m}$ were the lowest (Spotted Sicklefis, $n=0$; Sagor Catfish, $n=2$; Gray Eel-Catfish, $n=1$) at 0 %, 67 % and 33 % respectively. The abundance of microplastics that were of size $0.51 \mu\text{m}$ - $1.00 \mu\text{m}$ in Spotted Sicklefis, Sagor Catfish, Gray Eel-Catfish were 0 % ($n=0$), 0 % ($n=0$) and 100 % ($n=10$) and of size $1.01 \mu\text{m}$ - $1.50 \mu\text{m}$ were 0 % ($n=0$), 0 % ($n=0$) and 100 % ($n=7$). Microplastics that were of size $2.00 \mu\text{m}$ and above were determined to be 20 % ($n=2$), 20 % ($n=2$) and 60 % ($n=6$) respectively.

In water samples, the microplastics identified range from $0.35 \mu\text{m}$ - $3410 \mu\text{m}$.

Figure 5 shows that the majority of the microplastics found in water samples were in the size class of $0 \mu\text{m}$ - $0.50 \mu\text{m}$ (Jan-20, $n=4$; Feb-20, $n=5$; Mar-20, $n=8$) at 24 %, 29 % and 47 % respectively. Similar to the result for fish samples, the least abundant microplastics were found in the class size $1.51 \mu\text{m}$ - $2.00 \mu\text{m}$ (Jan-20, $n=0$; Feb-20, $n=1$; Mar-20, $n=1$) at 0 %, 50 % and 50 % respectively. Meanwhile, the abundance of microplastics that were of size $0.51 \mu\text{m}$ - $1.00 \mu\text{m}$ (Jan-20, $n=1$; Feb-20, $n=5$; Mar-20, $n=10$) at 6 %, 31 % and 63 % respectively and of size $1.01 \mu\text{m}$ - $1.50 \mu\text{m}$ were 33 % ($n=3$), 11 % ($n=1$) and 56 % ($n=5$). Microplastics that were $2.00 \mu\text{m}$ and above were 33 % ($n=1$), 0 % ($n=0$) and 67 % ($n=2$) respectively. The microplastics in fish samples were similar in size to those in surface waters. This may be due to the sinking and resuspension of microplastics between sediment and surface water.

There are no existing standardized size criteria as size is a very common parameter in microplastic studies. It can be said that every research journal has presented a different class size, making it difficult to cite. Although plastic particles undergo mild fragmentation in freshwater settings than marine settings due to less wave action and turbulence, they will still undergo thermal and photo-oxidation by UV exposure to become smaller particles [12].

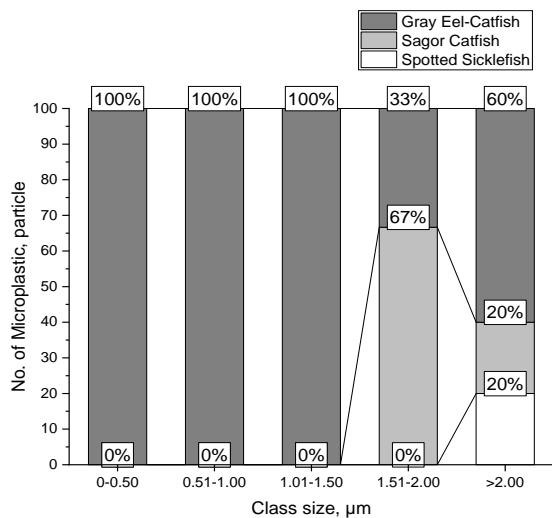


Figure 4. Size category of microplastics in fish species.

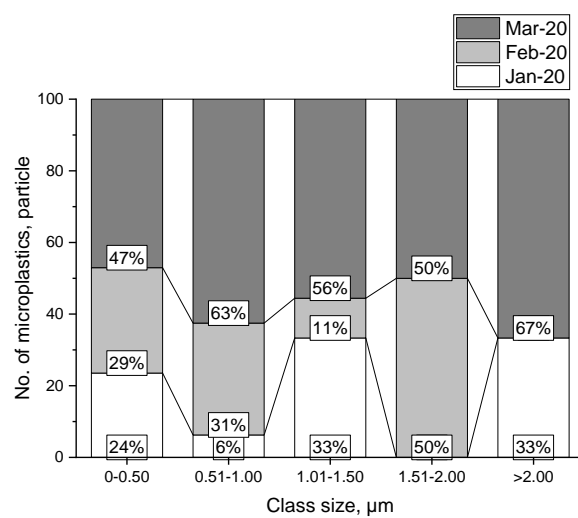


Figure 5. Size category of microplastics in water samples.

3.3. Shape of microplastics

Based on Figure 6, the percentage of occurrence of microplastic was 100 % fibres for Spotted Sicklefish, 100% fibres for Sagor Catfish. In Gray Eel-Catfish, the numbers of films found were higher than that of fibres at 47 % fibres and 53 % films.

Films originated from the degradation of plastic bags [28]. The presence of films indicates poor waste management. From Figure 7, the percentage of occurrence was 56 % fibres and 44 % films for Jan-20, 33 % fibres and 67 % films for Feb-20 and 12 % fibres and 88 % films for Mar-20. Figure 8 shows the shapes of microplastics present in the samples which consists of different coloured fibres and films.

Low-density microplastics can be present in the sediment zone due to biofouling. Biofouling causes the accumulation of foulants on the microplastics' surface which increase their density and cause them to sink and settle in the sediment layer [12]. Fibres are the most common microplastic type observed in sediments [7,27]. These fibres can be resuspended due to several reasons such as weather, bioturbation, tidal forcing, and trawling activity [29].

Fibres may originate from the use of fishing equipment as fishing is the main source of economy of the area. As the area of study is located near residential areas, fibres may also come from domestic effluent [28]. More than 100 fibres can originate from 1 L of wastewaters from washing synthetic clothes and an average of 1900 microfibrils can be released in a single machine wash [13].

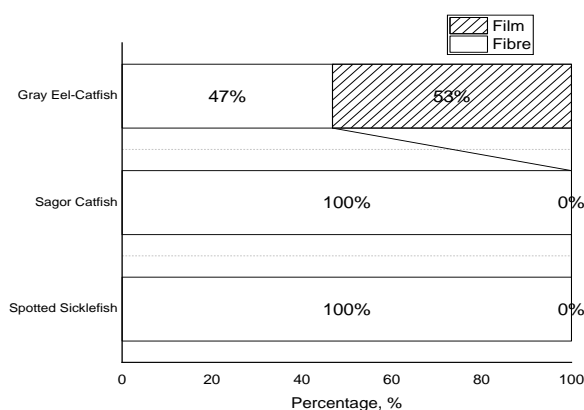


Figure 6. Percentage difference between shapes of microplastics ingested by fish species.

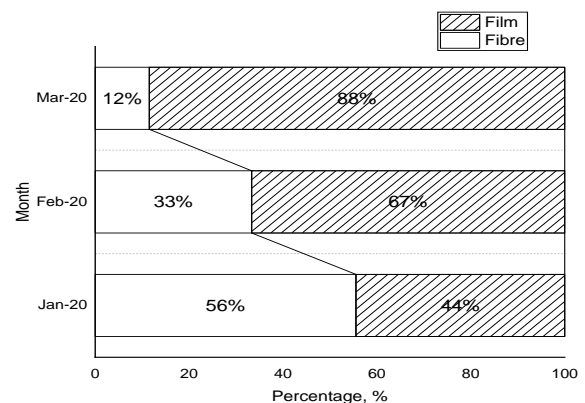


Figure 7. Percentage difference between shapes of microplastics in water samples.

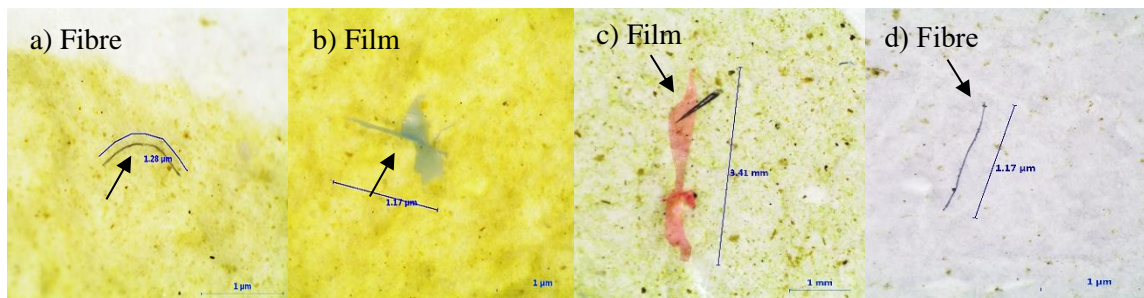


Figure 8. Microplastic in samples at 40x magnification: (a), (b) in fish; (c), (d) in surface water.

3.4. Colour of microplastics

Referring to Figure 9, the fish samples ingested mostly blue microplastics at 45 % ($n=24$) and the least amount of microplastics in yellow colour at 2 % ($n=1$). Amberstripe scads (*Decapterus muroadsi*) showed a preference for blue microplastics as the microplastics resembled their common prey which is the blue-coloured copepod species. This suggests that fish mistakenly ingested microplastics that resemble their natural prey [30].

In water samples (refer Figure 10), there is a very narrow scope for comparison with other worldwide researches since the microplastic colour depends on the majority of the colour of plastic used in the study area. Based on Figure 10, blue microplastics were determined to be the most abundant at 49 % ($n=23$). It is safe to assume that most residents in the study area used blue-coloured plastics in their daily routines followed by black-coloured plastics, red-coloured plastics, green-coloured plastics and transparent plastics. Thus, it can also be concluded that the abundance of ingested blue microplastics in fish samples came from surface water.

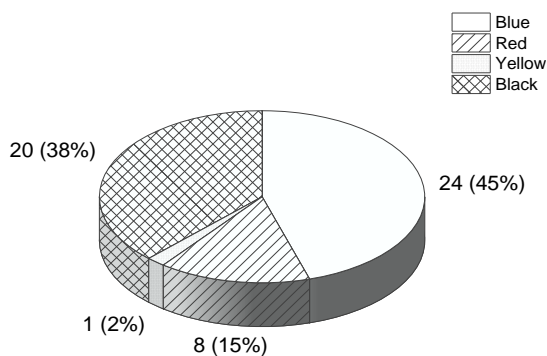


Figure 9. Colours of microplastics ingested by fish species.

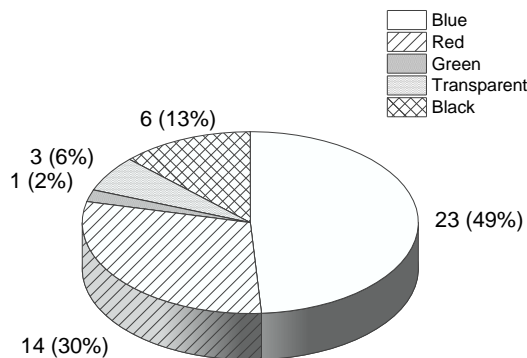


Figure 10. Colours of microplastics in water samples.

3.5. 3.6 ATR-FTIR analysis

Spectra produced from the microplastics were compared with a study by Jung et. al [31]. The adsorption bands used for identifications provide an almost perfect match to polyethylene (PE) and polyethylene terephthalate (PET). Fibres were determined to be PET whereas films were determined to be PE. PET and PE are common polymers used in the textile and packaging industry respectively [32,33]. Thus,

domestic effluent and improper plastic waste management can be pinpointed to the sources of the microplastics.

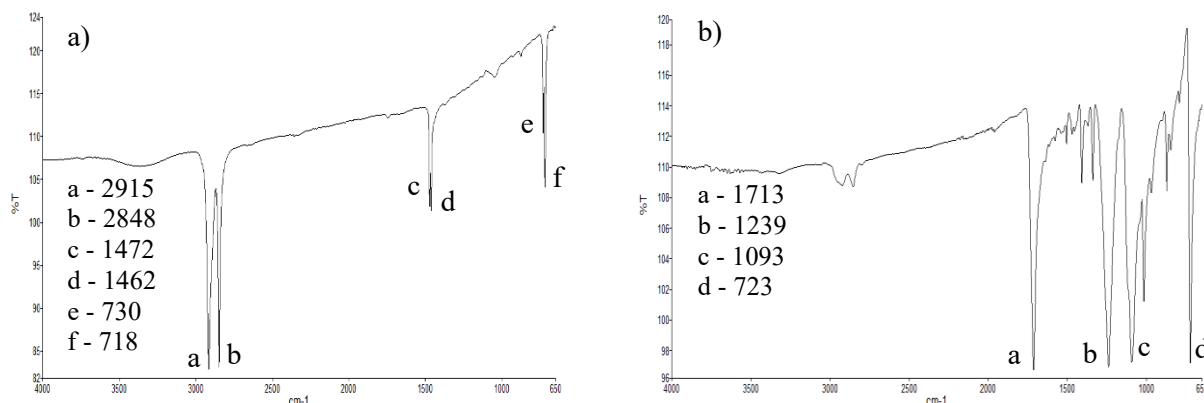


Figure 11. Spectra produced from microplastics: a) PE; b) PET

4. Conclusion

Microplastics were found in both Spotted Sickfish and Sagor Catfish, but only 6 out of 12 Gray Eel-Catfish species. All water samples contained microplastic which indicates that microplastic contamination is a problem in the study area. There were significant variations in the number of microplastics between Gray Eel-Catfish and the other two fish species due to differences in the number of specimens and GI tract size.

The water samples taken during March 2020 contained more microplastics due to possible bioturbation during mussels mating season. Overall, there were more fibres in fish species and more films in water samples. Microplastics can sink and resuspend in the water-sediment zone.

The abundance of blue microplastics in the water samples may be due to the typical blue plastic bags used in the study field and the preferences of fish towards different colour microplastics suggest accidental ingestion towards microplastics similar to their natural prey. It is safe to assume that the surface water contributes to the abundance of ingested microplastics by the fish samples. Unfortunately, the exact source of these microplastics is unknown. However, they are most likely to be present due to improper plastic waste disposal, domestic effluent that flows into the river body, and the fishing activities in the study area. Further study should be done to assess microplastic content in different organs of fish to investigate the translocation of microplastics in fish which may impose harm to humans upon consumption. Overall, the microplastic pollution level can be assumed to be at a low level. However, it is always important to practice proper plastic waste disposal and be knowledgeable in what food we consume as microplastics can bioaccumulate in aquatic animals.

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