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Investigation of Macro, Meso and Microplastics in Fish Gut from Coastal West Coast of Sabah, Malaysia

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Abstract Over the last decade, the production of plastics has increased with their increasing usage. Plastics have been seen as the most widely encountered waste in the environment. However, little is known regarding the accumulation of MPs in different tissues of fishes, especially in seawater in natural environments. In this study, the abundance of macro, meso and microplastics in guts from pelagic, demersal, and benthic groups were examined. A total of 70 individual fish guts from seven species (fish per species n = 10) were examined. These groups were chosen based on their distinct habitat features which lie in their preferred depth and location within the water column. Samples were taken from fish markets in Tuaran, Menggatal, Lido, and Kota Kinabalu, Sabah Malaysia. Remarkably, this study found meso and microplastic from the 2 pelagic species which are *Seriola rivoliana* and *Scomberomorus commerson*. In terms of characteristics, the polyethylene terephthalate (PET) particle was found in the form of fragment, fiber, and sphere while the polystyrene was in the form of fragment. Our results provided useful information for the assessment of the environmental threats posed by microplastics in Sabah, with a focus on the perspective of marine organisms.

Keywords Plastic, Pelagic and Benthic Fish, Polymers, Marine Environment

1. Introduction

Plastics have been an important aspect of human life since its first production. Nowadays plastics are always chosen over traditionally used materials such as ceramics, glass, metals, and wood because of their high durability and because they are not susceptible to loss of strength when they get wet. The usage of plastics can be seen in packaging, medicinal, industrial, and construction applications. Items like plastic bottles, bags, and containers are frequently used for storing and transporting goods [1]. In the construction sector, plastics are employed in a multitude of ways. They are used in the creation of pipes for plumbing systems, insulation materials for energy efficiency, and roofing materials that offer durability and weather resistance. Composite plastics are also utilized in structural elements, adding strength and longevity to construction projects. However, most of the plastics that are being used today are of single-use plastics such as plastic bags and disposable plastic containers which have low-value recovery and are difficult to degrade [2]. With the undisciplined attitude of our society coupled with few environmental factors, these plastics have made their way into the environment and cause pollution.

The presence of plastics in marine debris is largely contributed by human activities. Activities such as illegal dumping and indiscriminate disposal of wastes have caused the accumulation of plastics in the environment. Most plastics are reusable due to their high durability.

However, not many users are reusing their plastics, and this has led to the accumulation of plastics as waste. These plastics are lightweight; hence they can be easily transported from one location to another. Once they end up in the environment such as in the landfill, they can be easily blown by the wind to other locations such as the ocean or any water bodies. The most commonly used plastics such as polyethylene do degrade through chemical and physical weathering but at a very slow rate and it can take up to hundreds of years for it to be completely degraded. Before their complete degradation, these plastics will be fragmented into smaller sizes of macro, meso, and microplastics. These small sizes of plastics can cause even more serious harm to the environment, especially to living organisms with microplastics being the cause of concern as ingestion has been observed in a wide range of marine organisms [3]. The small sizes of microplastics enable them to be easily ingested by a wide range of organisms in the marine environment. Studies have shown that the intake of microplastics in fish is affected by many factors, such as local microplastic pollution levels, fish nutrition levels and feeding strategies [4].

In the marine environment, the fate of this plastic debris depends largely on their density which influences their buoyancy in seawater, position in the water column, and the possible interaction with marine biota [5]. Their density is determined based on the polymer type of the plastics debris and those with density greater than that of seawater will sink down to the bottom of the ocean. Plastic debris with low density will float on the surface of the ocean. However, over time, the low-density plastic debris can sink too when its density increases through biofouling by marine organisms.

Due to their small and microscopic sizes, it is easy for these plastics to enter our water system as they can easily pass through the filters in water treatment plant. Besides their aesthetic impacts, the presence of marine debris in the marine environment also poses direct threat to marine organisms through accumulation, choking, suffocation, entrapment, and entanglement [6]. Once in the marine environment, these plastics debris pose threats to marine wildlife because they can be easily mistaken as phytoplankton due to their small sizes. When microplastics are ingested by marine organisms, they can cause chemical and physical harm. Besides that, these plastics debris can be toxic to marine organisms due to their ability to collect and transfer pollutants such as heavy metals, and persistent organic pollutants (POPs) from the water to the organisms through ingestion [7]. Marine debris acts as an indirect source of organic contaminants to marine organisms when they mistake the debris for food [8].

Microplastics are described as being similar in size to sediments, which are small particles of rock, sand, or organic matter that settle at the bottom of bodies of water. This similarity in size makes microplastics difficult to distinguish from natural sediments [9]. Several factors can affect the bioavailability of microplastics to fish. Due to their non-selective feeding habits, filter-feeding and deposit-feeding fish are believed to be more susceptible to microplastics ingestion than predator fish [10].

The focus of this study will be the investigation of different sizes of plastics in pelagic, demersal, and benthic group fishes collected from supermarket area Kota Kinabalu and Tuaran, Sabah Malaysia. The types of fish used in this study were chosen because they are extensively consumed by humans as a direct source of protein in their diets. The study was done by extracting and isolating macro, meso and microplastics from the fishes and determining the polymer types found in the guts.

2. Methods

2.1. Sample Collection

In this study, pelagic, demersal, and benthic fishes were selected based on their ecological roles and feeding behaviors, along with their popularity in human consumption, to ensure they had the potential to accumulate microplastics [11]. Fish samples of 7 species were purchased from the fishery markets of Kota Kinabalu and Tuaran Sabah, Malaysia.

The 4 pelagic and demersal fishes (Figure 1) and 3 benthic fishes (Figure 2) were purchased from local fisherman who collected fish on a saltwater West Coast of Sabah. The taxonomic identification, the standard length (mm) and weight (g) of each fish individuals were determined and measured immediately. After that, the labelled specimens were transported to the laboratory and frozen at −20 ℃ before further analysis [6]. A total of 70 adult individual fish were purchased to study their feeding preferences of microplastics. The samples were prepared and dissected for isolation and identification of macro, meso, and microplastics. Three individuals of approximately equal length were selected for each species.

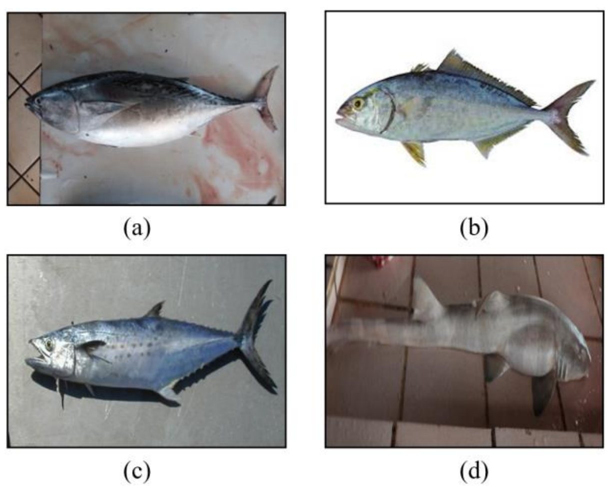


Figure 1. Pelagic fishes (a) Euthynnus affinis (b) Seriola rivoliana (c) Scomberomorus commerson and demersal species (d) Elasmobranch

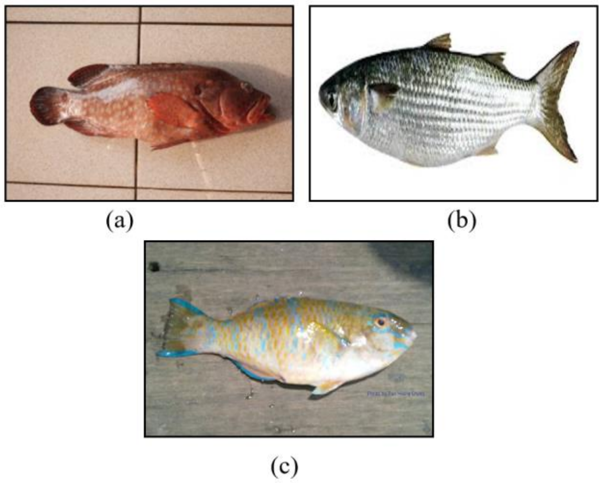


Figure 2. Benthic fishes (a) Cephalopholis cyanostigma (b) Liza subviridis (c) Scarus ghoggan

2.2. Quality Control of Experiments

To decrease the possibility of contamination, all apparatus (e.g., glass wares and dissection instruments) were cleaned three times with filtered water [12]. During the experiment conducted, gloves and lab coats were worn, and all samples were covered with foil paper. Control samples were processed at the same time of gut samples following the described protocol [13]. The experimental procedures without tissues samples were carried out as blank experiments.

2.3. Dissection and Isolation of Plastics from Fish Guts

All the fish guts were dissected and isolation of plastics from each fish gut was done in different beakers. For organ specific plastic analysis, 7 species with fish gut were selected. The stomach and intestine of each individual were removed, and three replicates of stomachs and intestines were used for analysis in each species to reduce the error within groups. The shape, size and internal structure of the stomach and intestine were observed and recorded (Figure 3). Extraction of plastics was carried out according to the method described by Romeo [14]. To improve the efficiency of plastic extraction and characterization from tissue, the gut was treated with a digesting technique. Roughly 200-400 mL of 30% hydrogen peroxide (H_2O_2) was added to digest the organic matter of the samples [15]. Bottles were covered and placed in an oscillation incubator at 65℃ and 80 rpm for 24-72 hours (depending on digestion level) to ensure appropriate digestion.

The isolation of plastics was carried out using floatation method by adding sodium chloride solution with density of 1.2 g/cm^3 into each beaker. The solution was left for two to three hours to allow the floatation of particles which were less dense than 1.2 g/cm^3[15]. The solution was then filtered using 0.45 µm (Whatman) membrane filter paper on a filter housing connected to a vacuum pump. The membrane filter paper was then dried in the oven at a temperature of 40℃ and placed under an inverted light microscope to analyze the composition of the plastics retained on the filter paper. The plastics were then differentiated according to their sizes where macroplastic is more than 25 mm, mesoplastic is between 5 and 25 mm, and microplastic is less than 5 mm [16].

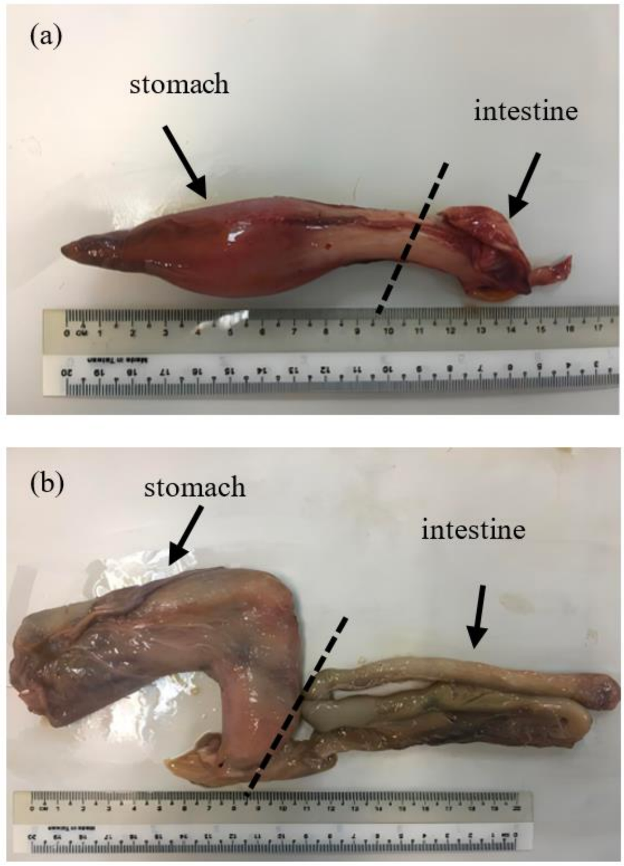


Figure 3. Fish guts of (a) Scomberomorus commerson (b) Seriola rivoliana

2.4. Identification and Validation of Microplastic Polymer

A Fourier-Transform Infrared (FTIR) spectrometer (Model: Brucker was utilized to identify and quantify microplastics in the samples. The samples were then scanned in 4000–450 cm^{-1} wave number [7]. The data were present in the form of spectrum peaks output from the scans expressed as %T. The results were identified with online polymer spectra databases from the libraries. For morphological features, the plastic fragments were observed under a stereo microscope and a Carl Zeiss Scanning Electron Microscope (SEM). Images were captured and then enhanced for suitable brightness and contrast.

3. Results & Discussion

3.1. Abundance of Macro, Meso and Microplastics in Fish

A comparison of the percentage of plastics recovered from guts of pelagic, demersal and benthic fishes was carried out by quantifying the number of plastics found in each fish species (Table 1). In this study, only two fish species were identified to have ingested plastics. The percentage of plastics debris recovered from the guts of *Seriola rivoliana* species was found to be 13% which is the highest among all the fish species sampled for this study where the recovery was 5-fold as compared to the recovery in *Scomberomorus commerson*. Besides *Seriola rivoliana*, plastics debris was also recovered from the gut of *Scomberomorus commerson*. In total, six plastic particles were recovered from the guts of these two species.

Table 1. Number and percentage of plastics recovered from the guts of randomly selected pelagic and benthic fishes

Habitat	Fish species	Number of plastics recovered	Percentage of plastics recovered (n=38)
Pelagic	*Seriola rivoliana*	5	13%
	Scomberomorus commerson	1	3%
	Euthynnus affinis	ND	ND
Demersal	*Elasmobranch*	ND	ND
Benthic	*Cephalopholis cyanostigma*	ND	ND
	Liza subviridis	ND	ND
	Scarus ghoggan	ND	ND

Among of 6 recovered plastics, 4 of them are classified as microplastic and 2 as mesoplastics. Pelagic feeding species were found to have ingested a statistically higher number of plastic particles compared to benthic species. This result confirms the study done by Deudero and Alomar [17], reporting that the ingestion of meso and macroplastics by benthic species is rarely reported and is infrequent. Previous studies by Lesher et al. [18], reported that fish can consume fouling and high-density plastic materials through prey. Plastic ingestion is most likely occurring during fish feeding activities. Feeding behaviours and habitat play crucial roles in trash ingestion, and an increase in plastic quantity enhances plastic bioavailability. The higher abundance could be attributed to various fish habitats and the amount of plastic waste along the seafloor [19]. Romeo et al. [14] reported the first evidence of presence of plastic debris in the stomach of large pelagic fish in the Mediterranean Sea. Longfin yellowtail also known as *Seriola rivoliana* is a benthopelagic fish that is circumglobally distributed with a high commercial value and interest in sport fishing [20]. Benthopelagic refers to marine organisms which live and feed near the bottom as well as in midwaters or near the surface.

The occurrence of plastics in the guts of these two species could be explained by their feeding strategy of this species. *Seriola rivoliana* can be found in waters from 3 to 250 meters deep. This species is a fast-swimming predator that feeds both day and night mostly on other fish as well as shrimps, and squids. Plastic particles could be ingested by this species during the predation action when they hunt fish. There is also a difference between the behavior of young and adult *Seriola rivoliana*. The young can be mostly observed to be surrounding floating objects while the adults usually feed closer to the bottom of the sea [21]. Young *Seriola rivoliana* are more susceptible to plastic debris ingestion as these materials are mostly found floating on the surface of the ocean. Plastic debris, especially those that are in the form of beads, was found to be ingested by *Seriola rivoliana* as they resemble the spores that are taken up by this fish species.

Scomberomorus commerson is also a pelagic species which can be found on the continental shelf to depths of 100 meters. They are a shallow-water species and are occasionally found as deep as 24 meters. This species is an opportunistic carnivorous species and feeds throughout the water column on small fishes including shrimps and squids. Similar to *Seriola rivoliana*, this species could also ingest plastic particles during feeding.

Although most plastics are buoyant and float on the sea surface, there are reports of plastics sinking to the sea floor. The mechanism which aids the sinking of these materials below the sea surface could be due to bio-fouling. When these materials are present on the sea surface, they provide a surface for attachment of bacteria, algae, and phytoplankton. This increases the density of the plastics hence causing it to sink. The presence of phytoplankton on the surface of the plastics also tricks marine organisms into ingesting the plastics.

The absence of plastics in all the other fish species could be due to the short residence time of plastics within the guts of fish. Foekema et al. [22] reported the same result where the author found plastic in only 2.7% out of 1203 fishes sampled. Another report from the same study also reported that more than 80% of fish that ingested plastic contained only one particle. This report is similar to the result in this study where all 6 fish samples which contain plastics have only one particle each.

3.2. Polymer Type of Recovered Plastic Particles

The identification of the polymer type was carried out using FT-IR Spectroscopy because each polymer type of PP, PET, PS, and PE has different wavelength band. Besides that, different polymer types have different molecular and functional groups which can be identified by using this method. Table 2 shows that the two pelagic fish species have ingested plastic with various sizes and different types of polymers.

Table 2. Type of plastics recovered from the guts of *Seriola rivoliana* and *Scomberomorus commerson* fish and its sizes

Fish species	Sample code	Type of Plastics Polymer	Size (mm)
Seriola rivoliana	SrMP1	Polyethylene terepthalate	3
	SrMP2		2.5
	SrMP3		12
	SrMP4	Polystyrene	2.5
Scomberomorus commerson	SMP1	Polyethylene terepthalate	16
	SMP2		1.9

The type of polyethylene terephthalate (PET) particle recovered was in the form of fragment, fiber, and sphere while the polystyrene was in the form of fragment. This is similar to other studies where the plastic particles recovered from marine samples are also in the form of fragment, fiber, and spheres [18]. Polyethylene which has a density range of 0.98 g/cm^3 to 0.98 g/cm^3 is less dense than the seawater hence it can be found floating on the ocean surface. Based on the findings, there were 2 types of polymers found in guts of *Seriola rivoliana* which were classified as polyethylene terephthalate (PET) and polystyrene (PS) while for *Scomberomorus commerson* the polyethylene terephthalate (PET) was found in a gut (Figure 5).

In this study, the recovered PET (Figure 4a) is suspected to be PET resin pellets. The recovered PET plastic has a distinct greenish colour and is in the form of fragment indicating that this plastic is a result of fragmentation from macroplastic (Figure 4c). Typically, these pellets are clear or translucent and are used in the production of plastic packaging such as bottles and containers [23]. Besides that, PET is also used as synthetic fiber called polyester in fabric applications [24]. This shows that Figure 4b is a synthetic fiber made of PET. The density of the plastics also plays a role in its fate in the marine environment. PET which has a density range of 1.38 g/cm^3 up to 1.39 g/cm^3 can easily sink because it is denser than seawater. Meanwhile, PS has a density range of 0.011 g/cm^3 up to 0.032 g/cm^3. Figure 5 shows the FT-IR spectra for the plastics particles found from the guts of *Seriola rivoliana* and *Scomberomorus commerson.*

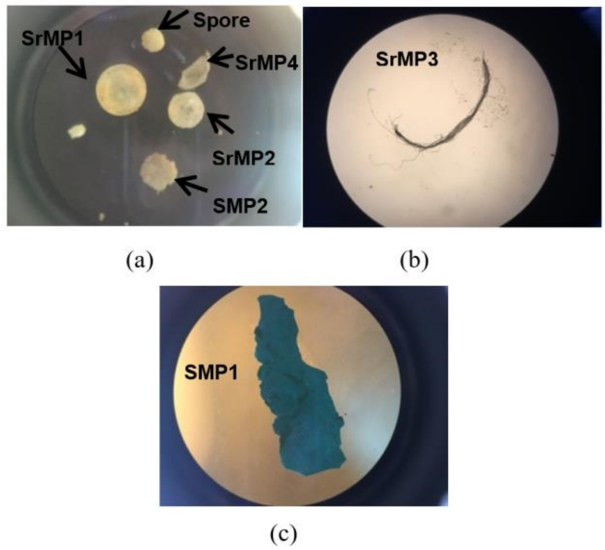
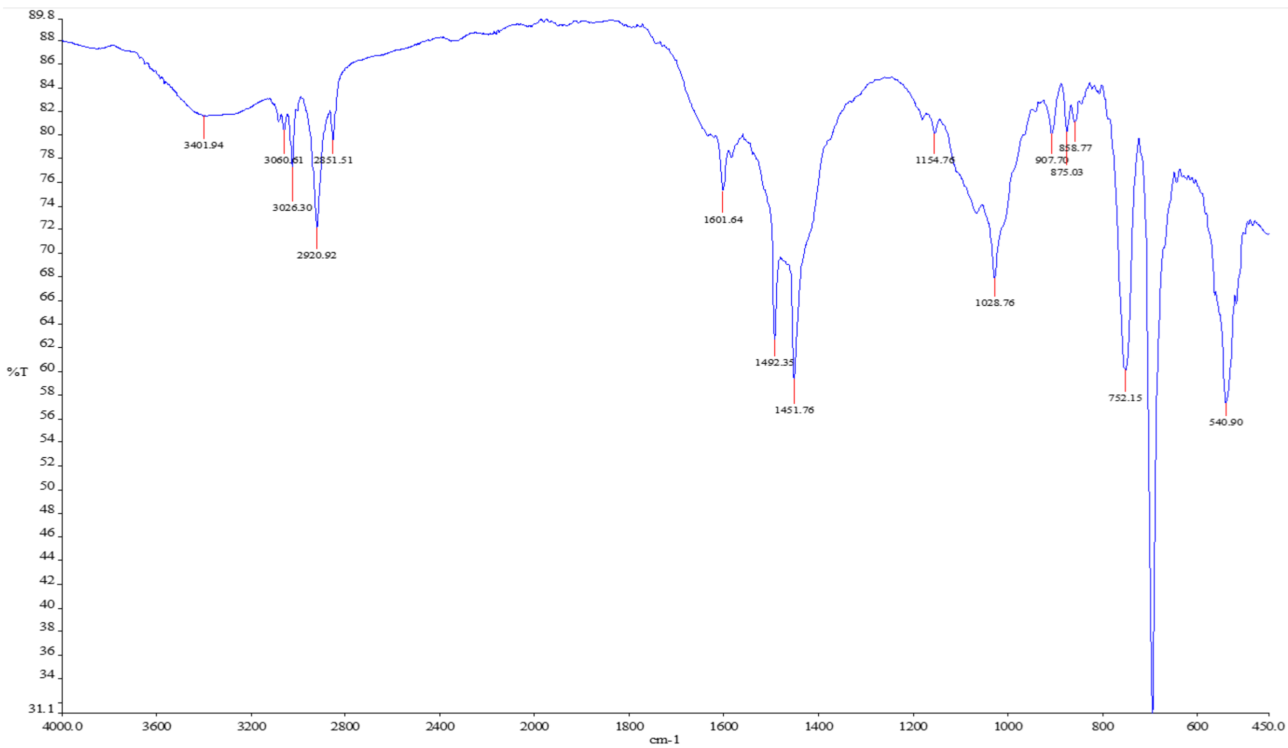


Figure 4. Plastic particles recovered from the guts of (a-b) *Seriola rivoliana* and (c) *Scomberomorus commerson* observed under a Scanning Electron Microscope (SEM)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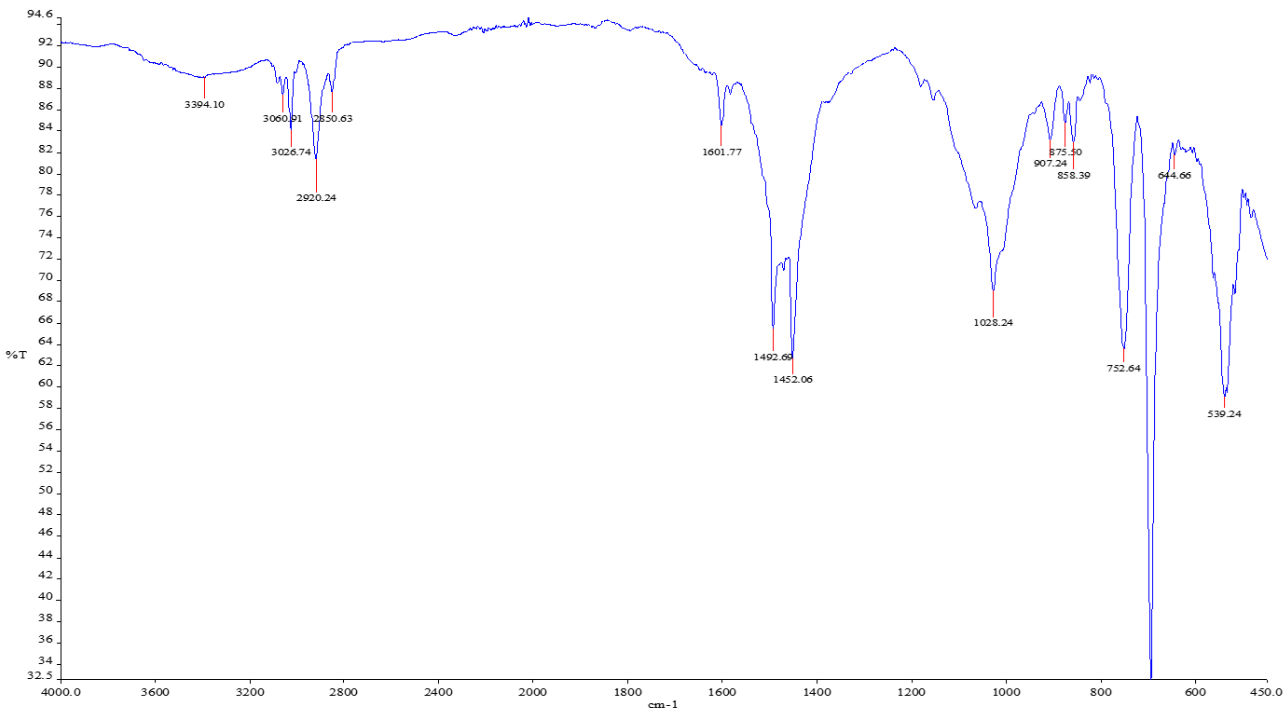
(a)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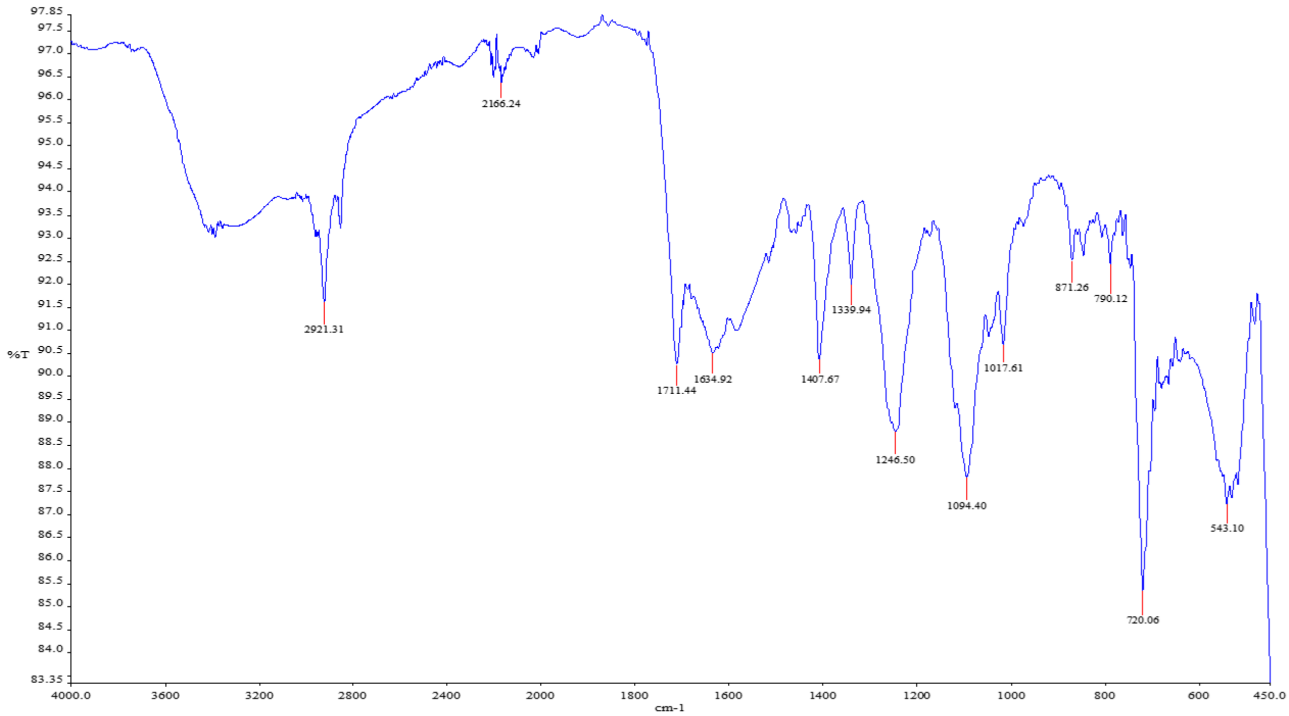

(c)

Figure 5. FT-IR spectra found in fish *Seriola rivoliana* (a-b) PET and PS while (c) PET found in *Scomberomorus commerson*.

4. Conclusions

In this study, we examined the accumulation of MPs in two species (pelagic species) while there was no plastics recovery from the guts of benthic and demersal fishes. The total number of plastic particles recovered was 6 among in which 4 were recovered from *Seriola rivoliana* and other 2 were recovered from *Scomberomorus commerson*. The size of the microplastics found ranges from 2.5 to 16 mm, with microplastic less than 3 mm being the most dominant. Polyethylene terephthalate (PET) is the most common polymer types found in the fish gut suggesting that it may have come from fishing gear or from consumer goods such as food packaging and mineral water bottles. We highly recommend studying a variety of species, habitats, and a larger sample size. Additionally, this will aid in identifying the origin of these microplastics and developing better approaches to address and manage plastic pollution.

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