



Baseline Study of Microplastics in the Gastrointestinal Tract of Commercial Species Inhabiting in the Coastal Waters of Karachi, Sindh, Pakistan

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A microplastics (MPs) emergence study in pelagic and mesopelagic species was carried out to delineate coastal degradation and ecosystem status around the Karachi metropolis. Species of high commercial and ecological worth were sampled using a gillnet of 1.5 cm knot-to-knot mesh size in November and December 2021. In total twenty-six individuals including *Liza subviridis* (15), *Thryssa dussumieri* (3), *Rastrelliger kanagurta* (2), and *Portunus sanguinolentus* (6) were used to perceive MPs. A strong linearity between body length and MPs ($R^2 = 0.937$, SE 0.071 and $R^2 = 0.928$, SE 0.104) were calculated for *L. subviridis* and *P. sanguinolentus*, respectively. However, the data of *T. dussumieri* and *R. Kanagurta* showed minimization failure. The MPs in GIT were extracted using direct observation under a sophisticated binocular microscope and chemical digestion (KOH) together with wet peroxide oxidation ($H_2O_2 + FeSO_4$) methods. The MP materials were categorized as foam, film, fiber, fragment, and beads of three different sizes 170, 120, 100 μm in the stomach, intestine, and esophagus. Film-type MPs appeared frequently, whereas beads were rarely seen. It is hoped that this baseline research would help to minimize industrial release, recognize critical knowledge gaps, and demonstrate MP flux being released into the aquatic environment. The results will support mitigation of this emerging threat to the living resources around the Karachi coastal area.

Keywords: microplastic, foodchain, seafood, contaminants, Karachi coast

INTRODUCTION

Overharvest of fisheries resources and climate change has already threatened marine ecosystems globally. In recent years the emergence of microplastics in aquatic systems has augmented such threats, particularly in developing countries. In Pakistan, approximately 0.2 million tons of plastic garbage has been discharged into the Arabian Sea by the coastal inhabitants and through the Indus River (Dawn News, 2019). The Indus River is considered among the most plastic-polluted rivers in the world (Mairaj et al., 2021). About 6,000 manufacturers are involved in producing 0.6 million tons of plastic in Pakistan (Dawn News, 2019). Regrettably, plastic materials account for 65% of total garbage in Pakistan, with a 15% rise predicted each year (WWF Pakistan, 2021). In the case

of a developing country, it is hard to figure out future plastic load likely to be dumped into the marine environment. Dealing with urban trash, Pakistan has encountered grave problems owing to the Karachi metropolis. Nevertheless, more plastic is anticipated to be dumped in the sea by 2050 (WWF Pakistan, 2021).

The emergence of microplastic would not merely degrade aquatic habitats but would also deform food web structures. The continuous surge of pollutants can lead to livelihood risk by affecting fisheries and aquaculture activities (Plastic, 2014; Barboza et al., 2019). In this context, Barboza et al. (2019) affirmed that various coastal areas receive 61~87% of their waste comprised of different forms of plastic. Nowadays the most widespread and persistent contaminants are plastics, which enter the coastal and marine environment by multiple routes comprising riverine and atmospheric transport, beach trash, and direct entry *via* aquaculture, shipping, and fishing (Lebreton et al., 2017; Villarrubia-Gómez et al., 2018). Gradually plastic waste is further worn into microplastics through processes of microbial degradation, extended UV exposure, and physical abrasion (Weis et al., 2015). The integration of marine litter steadily affects the coastal scenery, limiting recreational activities, causing a loss of touristic value, potentially harming the marine environment, and finally reaches the food chain. One of the primary concerns about the environmental consequences of microplastics is their interaction with the feeding of marine organisms (Botterell et al., 2019). As tiny as they are, plastics pose a problem for the environment since they are non-biodegradable and may find their way into marine food webs (Wright et al., 2013). Ingestion of microplastics, which may be mistaken for food and enter aquatic food webs, can occur by normal ventilation or swallowing them whole (Besseling et al., 2013; Setälä et al., 2014; Watts et al., 2014). Moreover, MPs may also cause indirect energy costs owing to their toxicity and tissue damage, and they may also be transmitted or enhanced *via* the food chain, presenting both ecological and human health hazards (Larue et al., 2021; Sokolova, 2021). Fish, bivalves, crabs, seabirds, phytoplankton, corals, and meiofauna have all been shown in many studies to bear adverse impacts of MPs accumulation (Wright et al., 2013; Mathalon and Hill, 2014; Watts et al., 2014; Van Cauwenberghe et al., 2015; Batel et al., 2016; Lusher et al., 2018).

In an aquatic ecosystem the food web plays a crucial role in energy shift among and within organisms through a series of organisms such as zooplankton to higher animals or grazers like fishes inhabiting the pelagic regimes. The entrance of MPs into the pelagic regime can shift up to the demersal organism as they prey on small organisms living around pelagic or demersal regimes.

Indigenous information on marine litter contamination is restricted to the abiotic environment (Balasubramaniam and Phillott, 2016; Qaimkhani, 2018; Tahira et al., 2020). Therefore, this study envisages the concentration of microplastic in the gastrointestinal tract of commercial and ecologically important species inhabiting around Karachi coastal waters. The findings will contribute to a better understanding of the propensity of microplastics to accumulate in fish and will help to fill a research

gap for future evaluations of the health-related risks and food security concern in Pakistan.

MATERIALS AND METHODS

Sample Collection, Transportation, and Laboratory Handling

A total of 26 specimens were sampled from pelagic and mesopelagic regimes around Karachi coastal waters (N 24° 52' 0.38 E 067° 00' 0.99) using a gill-net with a mesh size of 1.5 cm knot-to-knot during November and December 2021. Specimens were immediately placed in iceboxes and transported to the laboratory. After fishes were thawed at room temperature each specimen was identified carefully using FAO field guides/identification keys. Total body lengths were taken in centimeters and wet weight in grams. Species inhabiting pelagic and mesopelagic regimes of high commercial and ecological value were selected for MPs analysis. The gastrointestinal tract was extracted and poured into beakers for further analysis. The intensity of MPs accumulation in the stomach, esophagus, and intestine were further separated.

Avoiding Contamination

The digestion and oxidation of each gastrointestinal tract were chemically processed through potassium hydroxyl (KOH), ferrous sulfate (FeSO_4), and the solution of hydrogen peroxide (H_2O_2). The solution of 4M KOH and FeSO_4 was prepared by dissolving in powder/pellet in ultra-pure distilled water. These were placed on cycling vibrator HY-4C (0~350 rpm). For the separation of microplastics, each sample was passed through different sieves having mesh sizes of 170, 120, and 100 μm . Potential contamination during laboratory handling was minimized by the method adopted by Rasta et al. (2021). Glassware sterilization and cleaning was made using commercial dishwashing solutions to avoid contamination. These were further passed through ultra-pure distilled water, ethanol, and dried using an oven. The samples were further processed using a horizontal laminar flow cabinet to keep airborne MPs from mingling with it. To avoid contamination, the working surface was cleaned with 70% ethanol.

Microplastic Extraction and Analysis

Adopting a slight modification in the method used by Karami et al. (2017), MPs were extracted from the stomach, intestine, and esophagus separately. Here, we applied two types of treatments to extract the MPs from the GI tract. (i) Direct observation of microplastic in the stomach was made under a sophisticated binuclear microscope, but it was too vague to determine the type of microplastic. (ii) Digestion and oxidation of organic matter chemically treated was conducted to comprehend MPs and found to be a highly reliable approach. This approach allows us to examine intact plastic material without any damage.

Preparation of fixative was made with 4M KOH, H_2O_2 , and FeSO_4 . Solutions of KOH (4M w/v) and FeSO_4 were prepared by dissolving the content in ultra-pure distilled water. First, we

poured the stomach, intestine, and esophagus into 250 ml beakers separately. A blank control was adjusted to check for airborne contamination during experimental protocols. Precautionary measures were strictly applied during experimental work. A 30 ml (4M KOH) solution was added to each sample and placed on an electrical shaker for an hour at 350 rpm. After an hour intermission we added 5 ml of 30% hydrogen peroxide solution into each beaker and again placed it over the shaker for half an hour. Finally, the supernatant was filtered through a 125 μm sieve using a squirt bottle and particles collected back into the original beakers after a resting time of 2 h. Consecutively, wet peroxide oxidation for further extraction of MPs was applied. The reaction between the iron and oxygen turned the contents to a reddish-brown color. The contents were further passed through three sieves with mesh sizes 170, 120, and 100 μm . Consequently, contents were transferred into the corresponding Petri dishes for MPs identification and enumeration under a binocular microscope (Model: SMZ 1,100~Japan). As a first step, examining controls in empty Petri dishes was performed to rule out the possibility of airborne particle deposition in the laboratory. MPs were categorized according to their shapes, e.g., film, fiber, fragment, foam, and beads (Figure 1). The allocation and counting of plastic particles were maintained in separate containers and archived.

Statistical Analysis

Data of lengths and weights of each species were logarithmically transformed on a natural log. Mean, standard deviations, coefficient of determination, and standard error were calculated using excel spreadsheets.

RESULTS

This study envisages microplastic occurrence in the gastrointestinal tract (GIT) of the species of high commercial value dwelling in pelagic and mesopelagic regimes. A total of fifteen individuals of *Liza subviridis* (TL 16.3 ± 1.1 , TW 45.7 ± 6.8), *Thryssa dussumieri* ($N = 3$, TL 17.6 ± 2.3 , TW 46.83 ± 16.1), *Rastrelliger kanagartha* ($N = 2$, TL 15.8 ± 0.3 , TW 38.9 ± 1.4) and *Portunus sanguinolentus* ($N = 6$, TL 6.8 ± 0.66 , TW 84.8 ± 38) were used to observe MPs. Strongly linearity between body length and MPs ($R^2 = 0.937$, SE 0.071 and $R^2 = 0.928$, SE 0.104 was calculated for *L. subviridis* and *P. sanguinolentus*, respectively. However, data of *T. dussumieri* and *R. Kanagartha* showed minimization failure (MF). Surprisingly, no individual was found to be completely free from microplastic in the gastrointestinal tract, however, variation and frequency of occurrence were differing. It is interpretable from the coefficient of determination that there is a strong relationship between fish body length and MPs consumed by the nektons (Table 1).

Some 808 microparticles were counted from the GIT of *Liza subviridis*, *Rastrelliger kanagartha* (590), *Thryssa dussumieri* (330), and *Portunus sanguinolentus* (96). The lengths, weights, feeding habits, and habitat are summarized in Table 1. The frequency of occurrence of MPs indicates that fishes inhabiting the pelagic region ingest more suspended microplastic than that of the

mesopelagic regime. There is no recognizable pattern, though fish that ate more microplastic came from an area with higher ambient pollution. The occurrence of microplastic in three GIT parts (stomach, esophagus, and intestine) reveals that the plastic residues were categorically highest in the stomach and the lowest in the esophagus, this may be due to the low efficiency of digestion of plastic particles in the stomach where they accumulate and stay for a longer period (Figure 2). MPs were categorized as film, fragment, fiber, foam, and bead, based on their structure. Among them the frequency of film particles was the highest whereas bead-type particles were fewer (Figure 3).

A variety of shapes and colors of MPs in all gastrointestinal tracts was noticed, where transparent color particles were predominant. MPs particles were sieved in three sizes 170, 120, and 100 μm which were commonly found in all specimens. Nevertheless, particles measuring 170 μm were found significantly, whereas 120 μm were the lowest (Figure 4).

DISCUSSION

This is the first evaluation of the occurrence of microplastic (MP) particles ingested by highly economically valuable fishes and crabs caught by gill netter from the pelagic and mesopelagic regions of the Karachi coast. Overall outputs of this study have established baseline information that can be an input to underline an emerging dilemma in the coastal waters of Pakistan. The emergence of MPs in aquatic ecosystems seems a global problem. The consumption of MPs by microplankton, sessile organisms, and nektons can ultimately distress human health (Foley et al., 2018; Goswami et al., 2020). We found that both fishes and crab accumulated microplastics, although more accumulation of MPs was observed in the fishes rather than crabs; this might be due to dietary requirements and the fact that the stomach carrying capacity of fishes is larger than that of the crabs. Additionally, fishes constantly ingest seawater in search of food and ventilation, whereas crabs merely do so for ventilation. Wang et al. (2020) noticed that MPs accumulation is through more than the digestive tract, such as the hepatopancreas, guts, gills, and muscles. The gut and hepatopancreas components of the digestive system are the principal organs that chiefly function in digestion and absorption (Gibson and Barker, 1979). We observed that the fish esophagus, intestine, and stomach all contained MPs with varying frequency based on their holding capability, with the stomach having the most. This may be related to the inefficiency of plastic particle digestion in the stomach, where they aggregate and remain for a longer period. In this context, Ory et al. (2018) noted that microplastic particles are highly difficult for fish to digest, much more so than food. They added that MPs reside in the guts of *Saurida violacea* for a week on average, and up to 7 weeks, which is far longer than the time needed by fish to digest and egest food pellets, which is 2 days at most. Similarly, Gassel et al. (2013) calculated microplastic clearance rates in juvenile yellowtail, *Seriola lalandi* to a maximum of 4 days, and Hoss and Settle (1990). declared that in striped mullet, *Mugil cephalus*, MP clearance rate was up to a maximum of 10 days. Several studies have reported the higher

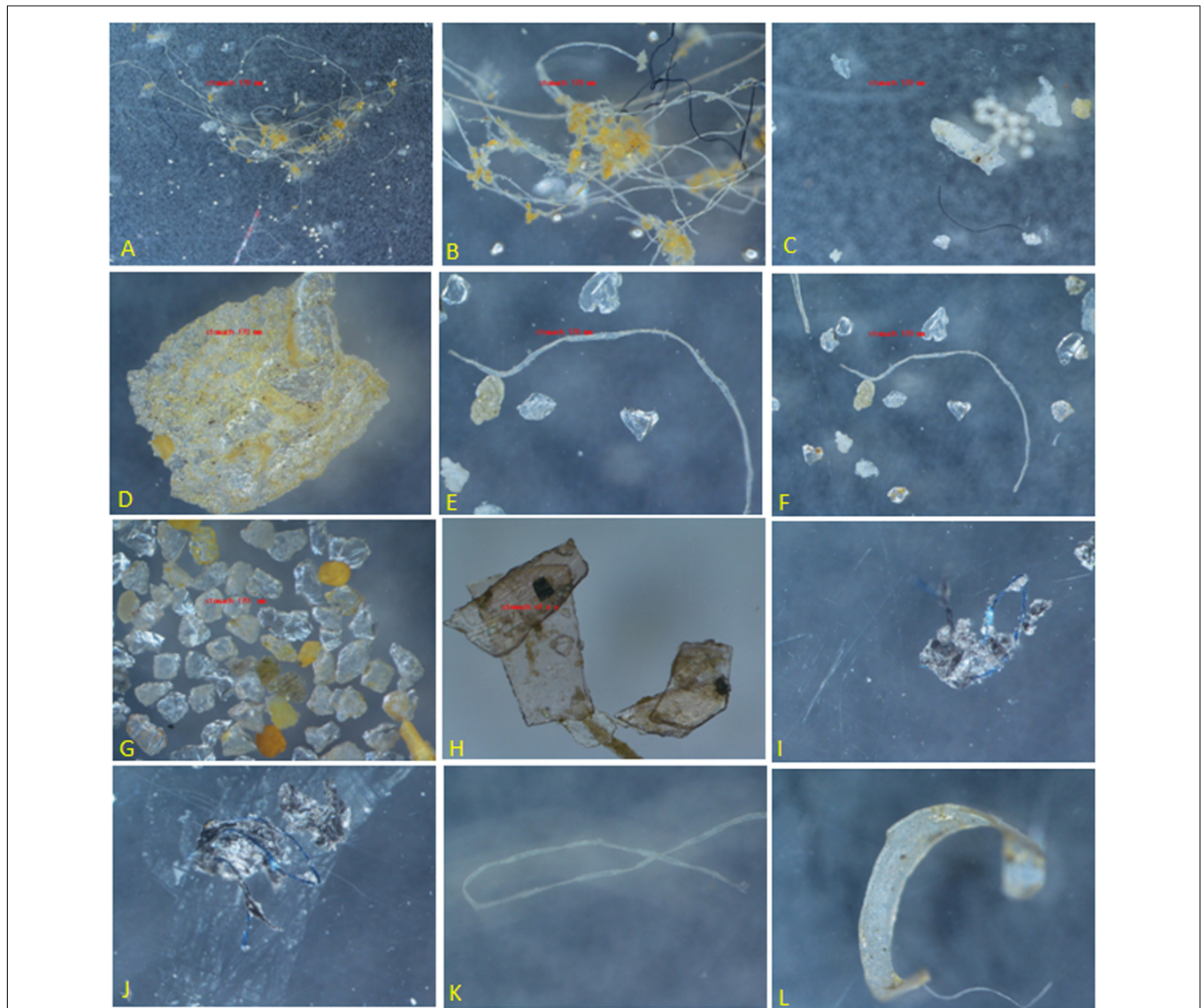


FIGURE 1 | Photomicrograph (170, 120, 100 μm) showing different shapes, sizes, and colors of microplastic (A–L) retrieved from the fishes and crabs inhabiting the waters around Karachi city (AG is beads; BEFKL fibers; CEFGLJ films; D foam and H is fragment).

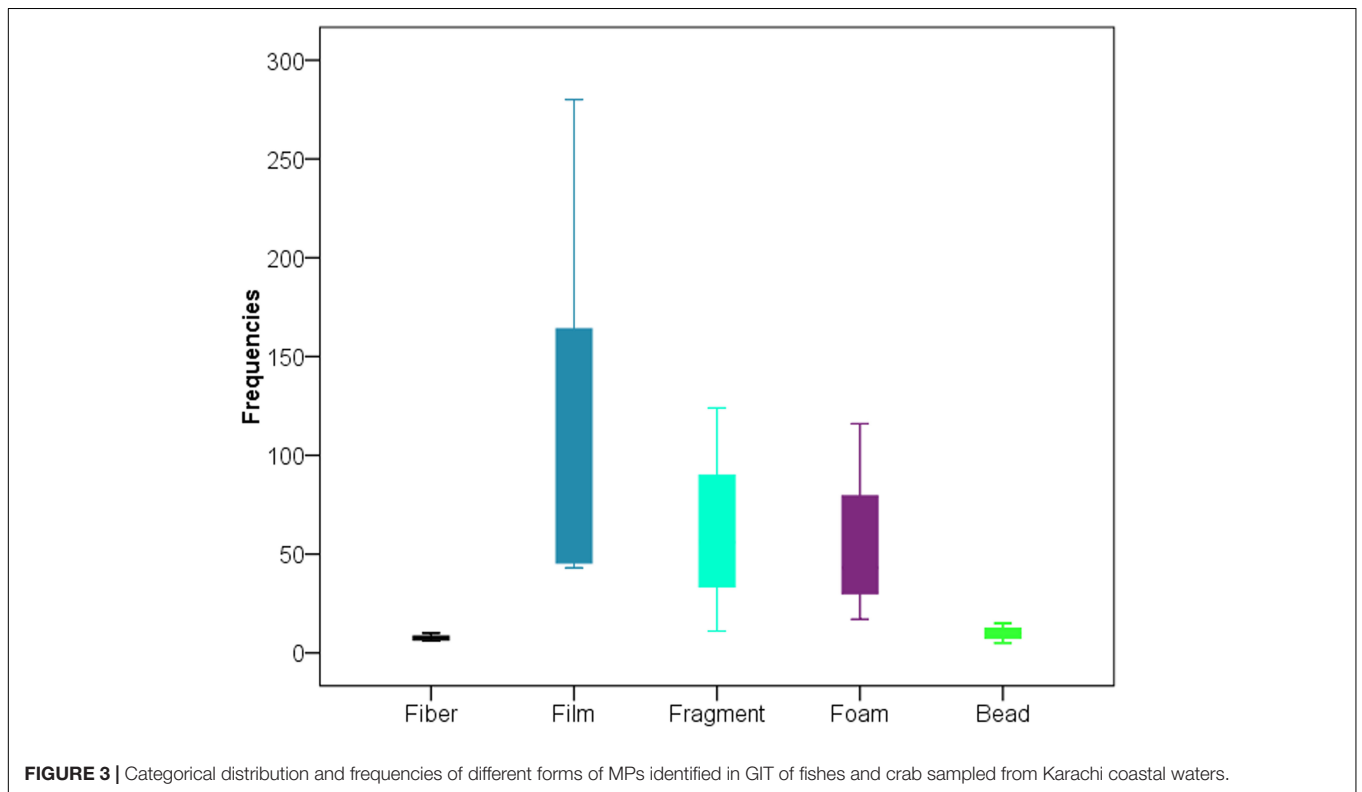
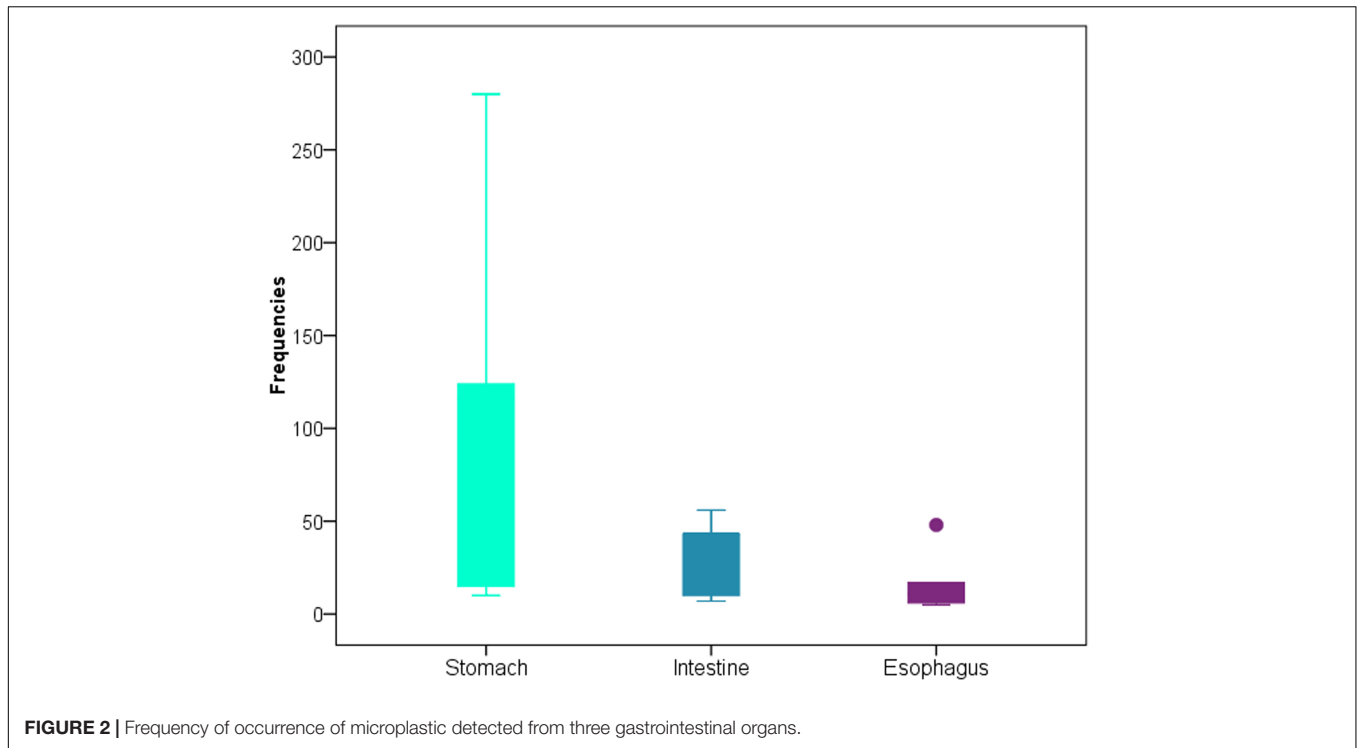
TABLE 1 | Summary of the morphometric characters, lengths, weights, feeding habits, and habitat of four species used to evaluate MPs in gastrointestinal tracts (N, number of specimens; TL, total length; TW, total weight; R², coefficient of determination; SE, standard error; MF, minimization failure).

Species	N	R ² (SE)	TLcm		TWgm		Stomach		Intestine		Esophagus		MPs	Feeding Type	Habitat
			Mean ± SD		W	L	W	L	W	L					
<i>Liza subviridis</i>	15	0.937 (0.071)	16.3 ± 1.1	45.7 ± 6.8	1.2	2.5	1.2	4.3	0.3	1.6	808	Generalist	Pelagic		
<i>Thryssa dussumieri</i>	03	MF	17.6 ± 2.3	43.83 ± 16.1	1.8	3	1.2	4.2	0.18	2	590	Generalist	Pelagic		
<i>Rastrelliger kanagurta</i>	02	MF	15.8 ± 0.3	38.9 ± 1.4	1.6	2.9	1	4.5	0.17	1.9	330	Opportunistic	Mesopelagic		
<i>Portunus sanguinolentus</i>	06	0.928 (0.104)	6.8 ± 0.66	84.8 ± 38	0.8	2.2	0.5	4.4	0.38	1.2	96	Opportunistic	Mesopelagic		

values of ingested MPs by the fish (Devriese et al., 2015; Peters and Bratton, 2016; Peters et al., 2017). In the northeastern Atlantic, there have been some reports of variations between distinct pelagic and deep-sea species (Pereira et al., 2020). Microplastic

was found in substantially higher concentrations in pelagic species than in deep-water species.

Various types of microplastics were recovered from the specimens, including films, fragments, fiber, foam, and beads.



Overall, the assessment reveals that film particles were the most common, whereas bead particles were rare in the gastrointestinal tract of organisms. Because films are secondary microplastics with weak polymers and low density, their microplastic

concentration is larger in water than in sediments. Humans often employ films to assist their everyday life, such as food/beverage packing, soap packs or detergents, plastic bags, and so on (Browne et al., 2011). Plastic packaging trash and high plastic

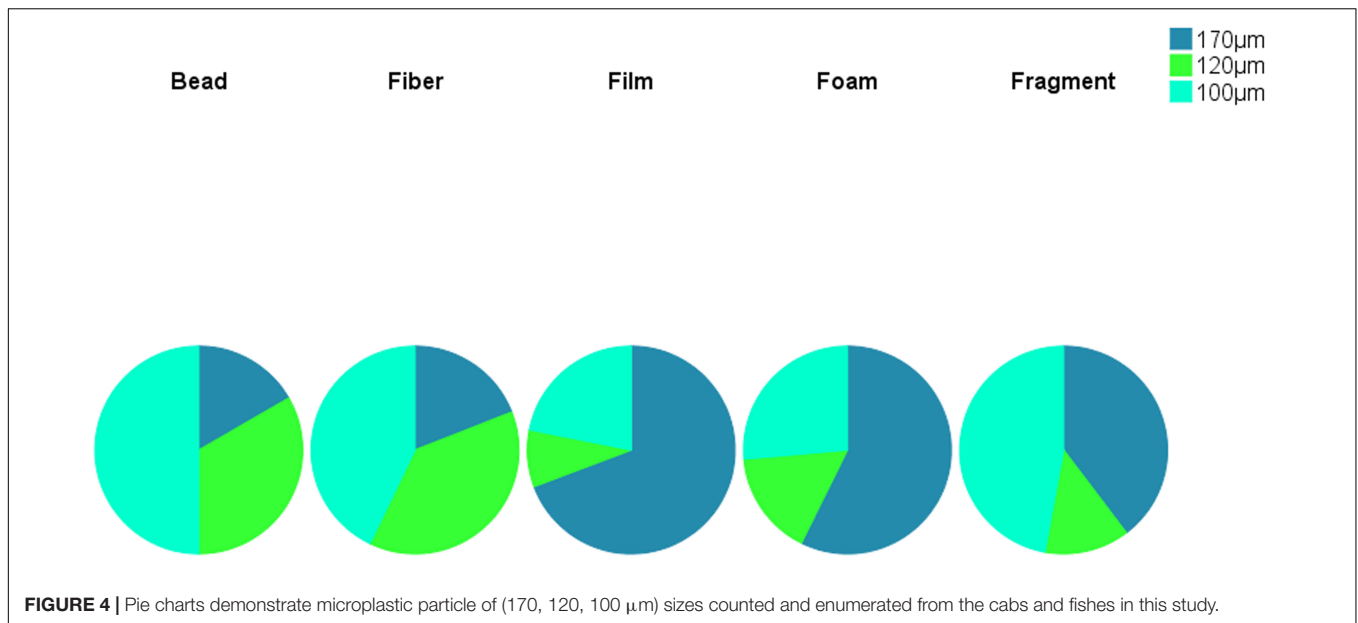


FIGURE 4 | Pie charts demonstrate microplastic particle of (170, 120, 100 µm) sizes counted and enumerated from the cabs and fishes in this study.

bag contamination in a region cause considerable biofilm growth over time, which is followed by changes in chemical-physical characteristics such as reduced buoyancy (Kershaw, 2015). Three sizes of MPs particles recorded in this study were common, this indicates that these types of plastic reach the aquatic ecosystem and chemically break into minute pieces that can easily be ingested by aquatic organisms. MP, which has a low density in comparison to seawater, tends to float on the water's surface. MPs, on the other hand, have a greater density than seawater and will sink and accumulate in sediments (Graca et al., 2017). However, we noticed a relatively lesser amount of MP fiber in the current study. Although, as Wright et al. (2013) pointed out, fibrous MPs are most prevalent in the aquatic realm.

Browne et al. (2011) mentioned that residential sewage including laundry waste may be a significant source of synthetic microfibers in the marine environment. The study area is in close vicinity of the metropolis of Karachi. Furthermore, the fisheries and shipping operations of the area are extensively established, and fishing may add to the occurrences of fiber in the marine ecology (Cole et al., 2011). It is worth mentioning here that the Karachi metropolis is closed to the sampling area can receive multiple inorganic pollutants. As a consequence, our findings may underestimate the overall concentration of MP fiber in the specimens. One possible explanation is that larger particles are more difficult for fish to eject and hence remain in their digestive tracts for extended periods of time. The longer the fiber, the more likely it is to get twisted in the gut wall and be difficult to eliminate from the body. Although, in the South China Sea, Zhu et al. (2019) studied microplastics in deep-sea fish and quoted that MPs are not limited to only the coastal ecosystem. Furthermore, Zhu observed no change in the contamination found from fish at different depths in terms of microplastics smaller than 1 mm in size, with film being the most abundant particle type followed by fiber and granules. Similarly, Wootton et al. (2021) reported

that the MP detected in Fijian fish was dominated by plastic film sheets. Thus, our conclusion is consistent with a previous study from the South China Sea and Fiji about the quantity of film in fish.

The use of chemicals in the textile and leather industries has had a negative impact on the soil, air, and occupational health of Pakistan. Pollution issues have emerged primarily as a result of the indiscriminate discharge of effluent from industrial and agricultural sources, as well as the dumping of untreated liquid and solid wastes created in the home into the coastal environment. Karachi has around 8,000 small and large industrial units that may be classified into several industrial zones. The export businesses, which transport their products *via* Karachi Port, generate a considerable portion of the coastal pollution. Much of the wastewater from these enterprises is untreated and discharged straight into the port area. The occurrence of MPs in the organisms is associated with the intensity of MPs present in the aquatic environment. The effect of microplastics on animal health and human health through the consumption of seafood is yet to be defined (Ory et al., 2018; Walkinshaw et al., 2020). Keeping in mind the availability of microplastic in all species described in this study validates that organisms around the Karachi coast have no choice but to consume microplastics. Our findings offer preliminary information that may be linked with future data to produce a comprehensive profile of microplastic contamination emergence in the ecosystem and seafood in Pakistan.

CONCLUSION

This study provides an insight into the microplastic emergence in some species of high commercial worth living in two domains (the pelagic and mesopelagic ecosystems) around Karachi coastal waters. This research can be an important contribution to our

knowledge and understanding of microplastic emergence in the ecosystem that can create human health associated issues by seafood consumption. We assume that particle size of $\leq 100 \mu\text{m}$ can be assimilated in flesh and other body parts. Similarly, we strongly agreed with Ory et al. (2018) who noted that MPs are much more difficult for fish to digest than that of large food particles, therefore, we suppose that particles of $\geq 125 \mu\text{m}$ size could stay in the stomach for a longer time. Generally speaking, untreated waste disposal and effluent discharge from fishing and shipping operations have all been suggested as potential sources of MPs in the marine environment. It is hoped that this baseline research would help to minimize untreated industrial release, recognize a critical knowledge gap, and demonstrate MPs flux being released into the aquatic environment will support mitigation of this emerging threat to the living resources around the Karachi coastal area.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories

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and accession number(s) can be found in the article/supplementary material.

ETHICS STATEMENT

Fish and crabs were sampled following the provincial code of conduct and HEC/CEMB/University of Karachi guidelines.

AUTHOR CONTRIBUTIONS

NA conducted the experiments and drafted the manuscript. SKP designed the research, supervised, and improved the quality of the manuscript.

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