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Original Article

Microplastics assessment in Arabian Sea fishes: accumulation, characterization, and method development

Avaliação de microplásticos em peixes do mar arábico: acumulação, caracterização e desenvolvimento de métodos

S. Riaz[a]* , S. Nasreen[a], Z. Burhan[a] , S. Shafique[a] , S. A. Alvi[b] and M. A. Khan[c]

[a]University of Karachi, Center of Excellence in Marine Biology, Karachi, Pakistan
[b]PCSIR Laboratories Complex, Applied Chemistry Research Centre, Karachi, Pakistan
[c]University of Karachi, Department of Zoology, Karachi, Pakistan

Abstract

Around the globe, plastic has been entering the aquatic system and is ingested by organisms. Identification, optimal digestion method, and characterization of the polymers to trace sources are of growing importance. Hence, the present work investigated microplastics accumulation, digestion protocol efficiency, and characterization of polymers with FTIR analysis in the guts of five fishes (*Lethrinus nebulosus*, *Rastrelliger kanagurta*, *Acanthopagrus arabicus*, *Otolithes ruber*, and *Euryglossa orientalis*) from the Karachi coastal area, Arabian Sea. A total of 1154 microplastics (MPs) were ingested by 29 out of 33 fish species (87%). The highest average MP/fish was recorded in *Otolithes ruber* (54) and the lowest in *Rastrelliger kanagurta* (19.42). Microfibers were the most abundant shape with the highest numbers (35.52%) as compared to the rest of the MPs identified. Transparent microfibers were recorded as the highest in numbers followed by red, black, blue, and green. In this study, KOH with different concentrations and exposure times along with oxidizing agent hydrogen peroxide was tested (Protocols 3 and 4). Results showed these bases were highly efficient in obtaining optimal digestion of the samples. FTIR analysis confirmed that the majority of the polymers found in the fish guts were polyethylene and polypropylene. This study validated for the first time the presence of these polymers of plastic in marine fish from Pakistan.

Keywords: FTIR analysis, polyethylene, Karachi coast, polypropylene, *Acanthopagrus arabicus*.

Resumo

Em todo o mundo, o plástico tem entrado no sistema aquático e tem sido ingerido por organismos. A identificação, o método de digestão ideal e a caracterização dos polímeros para rastrear fontes são de crescente importância. Portanto, o presente trabalho investigou o acúmulo de microplásticos, a eficiência do protocolo de digestão e a caracterização de polímeros com análise de FTIR nos tratos digestivos de cinco peixes (*Lethrinus nebulosus, Rastrelliger kanagurta, Acanthopagrus arabicus, Otolithes ruber* e *Euryglossa orientalis*) do litoral de Karachi, no mar Arábico. Um total de 1154 microplásticos (MP) foram ingeridos por 29 das 33 espécies de peixes (87%) estudadas. A maior média MP/peixe foi registrada no *Otolithes ruber* (54), e a menor no *Rastrelliger kanagurta* (19,42). As microfibras foram a forma mais abundante e com os maiores números (35,52%), em comparação ao restante das MPs identificadas. As microfibras transparentes foram detectadas em maior número, seguidas por vermelho, preto, azul e verde. Neste estudo, o KOH foi testado em diferentes concentrações e tempos de exposição, juntamente com o agente oxidante peróxido de hidrogênio (Protocolos 3 e 4). Os resultados mostraram que essas bases foram altamente eficientes para a obtenção de digestão ideal das amostras. A análise de FTIR confirmou que a maioria dos polímeros encontrados nos tratos digestivos dos peixes eram polietileno e polipropileno. Este estudo validou pela primeira vez a presença desses polímeros de plástico em peixes marinhos do Paquistão.

Palavras-chave: análise de FTIR, polietileno, costa de Karachi, polipropileno, *Acanthopagrusarabicus*.

1. Introduction

Ocean provides several valuable resources such as food, energy, and water as well as habitat for the marine ecosystem. There are various factors affecting the marine ecosystem including plastic pollution. Plastic pollution can alter marine ecosystems causing harmful effects globally (Urbanek et al., 2018). According to a study, as of 2010, 275 million metric tons of plastic have been produced by 192 coastal countries out of which 4.8 to 12.7 million metric tons entered the oceans (Jambeck et al., 2015). Production of plastic is expected to increase in the future

*e-mail: shagirajput7@gmail.com
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and probably double by 2025 (Lusher et al., 2017). Pakistan produces approximately 0.2 million tons of plastic debris going directly into the Arabian Sea (Dawn News, 2019). A study by Ali and Shams (2015) stated that plastic debris was about 45% of the total debris collected from the beaches of the Arabian Sea, Sindh, Pakistan.

Plastics both as primary objects or secondary smaller fragmented pieces called microplastics (size < 5 mm) enter the oceans mostly through rivers, sewage discharges, and land run-off (Moore, 2008; Barnes et al., 2009; Andrady, 2011). Marine organisms ingest microplastics (MPs) which ultimately moved across the food chain. Several studies have evaluated microplastic accumulation in marine species around the globe (Thompson et al., 2004; Avio et al., 2015a; Tanaka and Takada, 2016; Kumar et al., 2018; Sevillano-González et al., 2022) by the study of the targeted organ or entire organisms.

Digestion of biological matter is a crucial step for microplastic analysis and several protocols have been used and suggested by researchers (Pfeiffer and Fischer, 2020). Mostly quantitative studies are performed by using high-magnification microscopes. For qualitative studies FTIR analysis is used to get characterization of the polymers which led to the identification of the sources of microplastic.

FTIR come about to be the most popular technique worldwide for microplastics studies (Renner et al., 2018). It can identify different types of microplastics such as polyethylene and polypropylene which are the predominant type of plastics in many studies from around the world (Horton et al., 2017; De Lucia et al., 2018; Qu et al., 2018; Lenaker et al., 2019; Mani et al., 2019; Su et al., 2020; Kor et al., 2020).

Microplastics can serve as carriers for contaminants, pathogens, and metals from the aquatic environment into the organisms, causing toxic effects on the organisms (Alimba and Faggio, 2019). Many reported studies have shown the toxic effects of microplastics on organisms like reduced feeding (Murphy and Quinn, 2018) and immunotoxicity and neurotoxicity (Avio et al., 2015b) by polyethylene alone.

Thus, taking into consideration of this important problem of microplastic presence worldwide, we designed this study primarily to investigate the microplastic accumulation in marine fish species inhabiting shallow and deep waters in Pakistan. Moreover, this is the first attempt from Pakistan to characterize the identified microplastics from marine fish into polymers for indication of the sources of these plastics with the help of FTIR validation.

2. Materials and Methods

2.1. Sampling and contamination control

Five edible marine fishes were selected for this study. A total of 33 specimens were collected from the fresh landings at Karachi fish harbour in January 2022. Specimens were placed in iceboxes and transported to the laboratory and were kept at -20 °C until further procedure. Plastic-free material was used to avoid plastic contamination. Glassware equipment was thoroughly washed first with a dishwashing liquid and then with distilled water followed by rinsing with ethanol before oven-dried. To prevent any potential airborne microplastic contamination, the procedure was carried out in a laminar flow cabinet (Model: BBS-V500). Aluminium foil was used to cover beakers and other glass equipment during process. Before starting the procedure, the working surface was cleaned with ethanol (70%). Blank samples were prepared without fish gut in a 1:3 ratio (1 blank per 3 fish gut samples).

2.2. Fish sample treatment and digestion protocol

Each specimen was thawed, and total length (cm) and body weight (g) was measured before dissection. The gut was dissected out of the fish, weighed, and transferred into a 1000 ml glass beaker. For digestion, we tested different protocols and digestion solutions to reach a feasible and time-saving protocol. A total of four protocols were selected for this purpose and digestion solutions included, 10% KOH & 4M KOH, 1M NaOH, 30% H_2O_2 & 35% H_2O_2, saturated NaCl, and $FeSO_4$. Table 1 shows an overview of the digestion protocols tested.

2.3. Filtration and identification of Microplastics

After digestion samples were filtered through stainless steel sieves of three different sizes 300 µm, 150 µm, and 50 µm, and immediately shifted in Petri dishes. They were visualized under a stereomicroscope to identify and classify the plastic particles found according to their sizes, colours, and shapes. A hot needle test (De Witte et al., 2014) was performed for confirmation of plastic particles during the study. The hot needle was touched to the suspected particles and they started to curl. Microplastics were counted and classified according to their shapes in fragments, microfibers, beads, foams, and films. After visual examination, samples were vacuum filtered through Whatman filter paper (GF/C 47mm) and dried in an oven at 70 °C for FTIR analysis.

2.4. FTIR analysis

FTIR analysis was carried out using Agilent Carry 630 FTIR. Exposure time was 2 to 30 s, resolution 4, background scans 16, and spectra range 4000 – 650 cm^{-1}. All the acquired spectra were compared with the reference characteristic wave numbers provided in "Easy identification of plastics and rubbers" (Verleye et al., 2001).

2.5. Statistical analysis

Statistical analyses were performed by Statistical Package for the Social Sciences (SPSS, Version 22). The difference in MP particle abundance and total length between all species was assessed using an independent samples *t*-test. The mean values of MP and the length of the specimens were compared. MP/fish and MP/gram of weight of the specimen were calculated. A flow chart of the process of MP analysis is shown in Figure 1.

3. Result

3.1. Sample treatment

A total of 33 fish species were collected from Karachi Fish Harbour. Fishes were identified as *Lethrinus nebulosus* (n = 6), *Rastrelliger kanagurta* (n = 7), *Acanthopagrus arabicus* (n = 9), *Otolithes ruber* (n = 5), *Euryglossa orientalis* (n = 6). All the specimens had a length range between 21 cm to 48.4 cm and body weight between 137 g to 1420 g.

Procedural blanks were analysed with every batch of digested samples and there was no evidence of microplastic contamination. Digestion protocols 1 and 2 were observed to be time taking with less clear digests and degradation of MPs was also a concern. Nonetheless, protocols 3 and 4 were equally efficient in the complete digestion of the biological material. The only difference between protocols 3 and 4 was the conditional use of $FeSO_4$ which was used if harder structures such as shells and bones were part of the gut contents (Table 1). It provided extremely clear digests to observe under the stereomicroscope.

3.2. Microplastic occurrence and frequency

A total of 1154 microplastics were ingested by 29 out of 33 fish species (87%). The highest average MP/fish was recorded in *Otolithes ruber* (54) and the lowest in *Rastrelliger kanagurta* (19.42). Results of MP/g showed that the highest average MP/g was observed in *Euryglossa orientalis* (0.22) and the lowest in *Otolithes ruber* (0.04) (Table 2).

Microplastic shapes were identified in all the samples as microfiber, film, fragment, foam, and beads as shown in Figure 2. Microfibers were present highest in numbers (35.52%) as compared to the rest of the MP types while foam was observed to be the lowest (2.16%) in samples. Microfibers were found approximately the same in all the fishes except *Rastrelliger kanagurta* which had the highest number of beads in the gut while *Acanthopagrus arabicus* had the highest number of films, foam, and fragments as shown in Table 3.

Microfibers found in the samples were transparent, red, black, green, and blue. Figure 3 shows the composition of microfibers analysed in fish samples. Out of 410 microfibers observed in the samples, 42.19% were transparent and 28.04%, 17.80%, 6.58%, and 5.36% were red, black, blue, and green, respectively.

Table 1. Summary of the protocols used in microplastics analysis for digestion efficiency.

Protocols	Digestion Solutions	Concentration	Temperature °C	Exposure time	Equipment used
1	KOH	10%	60	5 days	Oven
	NaOH	1M			
2	H_2O_2	30%	60	2 days	Oven
	NaCl	Saturated			Centrifuge
3	KOH	4M	Room temperature	3 – 4 Hours	Orbital shaker
	H_2O_2	35%			
4	KOH	4M	Room temperature	3 – 4 hours	Orbital shaker
	H_2O_2	35%			
	$FeSO_4$				

Protocol 1: 10% KOH three times the volume of the sample was added to the beaker and kept at 60 °C in an oven for 5 days. Afterward, the sample was treated with 15 ml of NaOH and filtered. **Protocol 2:** 10 ml of 30% H_2O_2 was added to the beaker and kept at 60 °C in an oven for 2 days, centrifuged at 3000 rpm for 10 minutes, and filtered. 20 ml of saturated NaCl and centrifuged at 2000 rpm for another 10 minutes and filtered. **Protocol 3:** 30 ml of 4M KOH was added to the beaker and kept on the orbital shaker (KJ-201 BD) at 210 r/min for 1 hour. After keeping the sample at rest for 30 minutes, 5 ml of 35% H_2O_2 was added to the sample and kept on the shaker for another 30 minutes. After removal from the shaker, the sample was left for 2 hours and then filtered. **Protocol 4:** This protocol followed all the steps used in protocol 3 with an addition of $FeSO_4$ before filtration. It was used according to the gut content and feeding habits of the fish for complete digestion of hard shells and bones if any.

Table 2. Occurrence of average microplastics per fish individual and gram of fish weight.

Fish	Average TL (cm)	Average B.Wt (g)	Average MP/fish	Average MP/g
Lethrinus nebulosus	26.96	318	29.5	0.09
Rastrelliger kanagurta	22.24	148.28	19.42	0.13
Acanthopagrus arabicus	30.37	575.11	37.33	0.07
Otolithes ruber	47.1	1240	54	0.04
Euryglossa orientalis	24.58	186.66	39.16	0.22

Table 3. Frequency of different types of microplastics identified in fish guts.

Fish	Microfiber	Film	Foam	Fragment	Bead
Lethrinus nebulosus	92	31	3	13	38
Rastrelliger kanagurta	26	26	3	22	59
Acanthopagrus arabicus	93	96	16	71	60
Otolithes ruber	99	78	1	23	69
Euryglossa orientalis	100	50	2	24	59

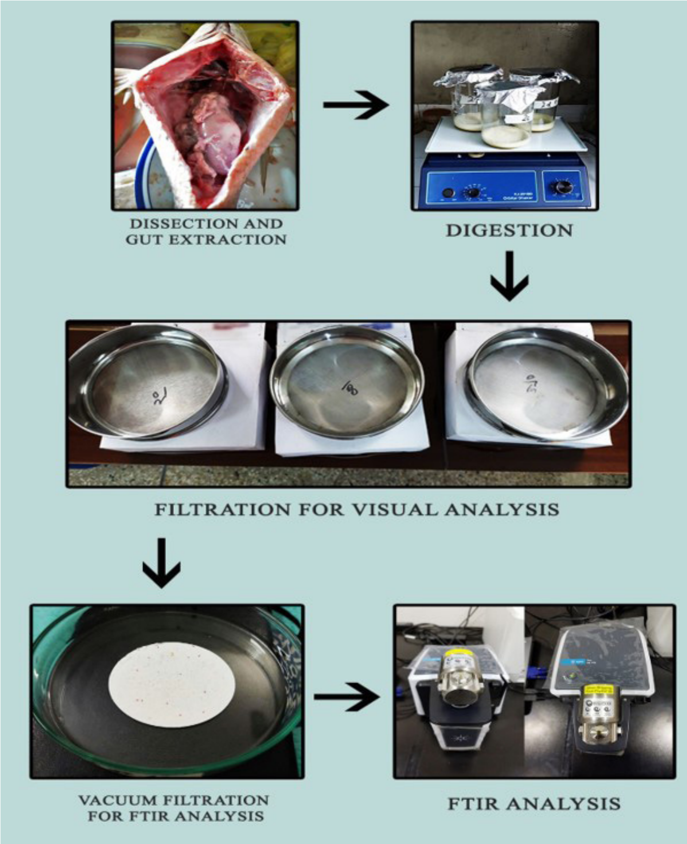

Figure 1. Flow chart of microplastic analysis with steps used in the process.

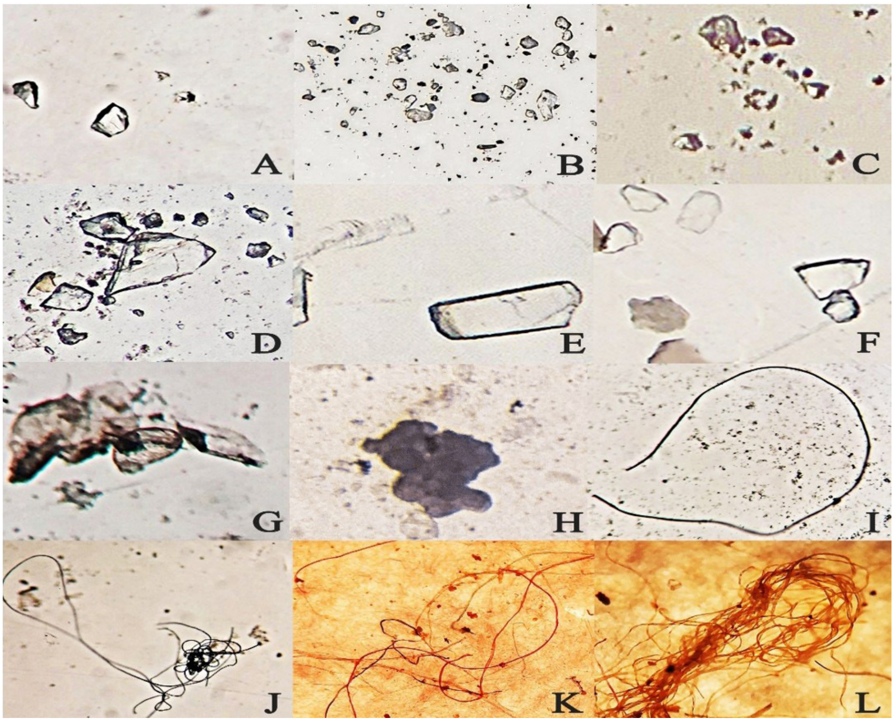

Figure 2. Types of microplastics identified as bead (A-D), film (B, E, F), fragment (G), foam (H), and microfibers found as single fiber and clump together (I-L).

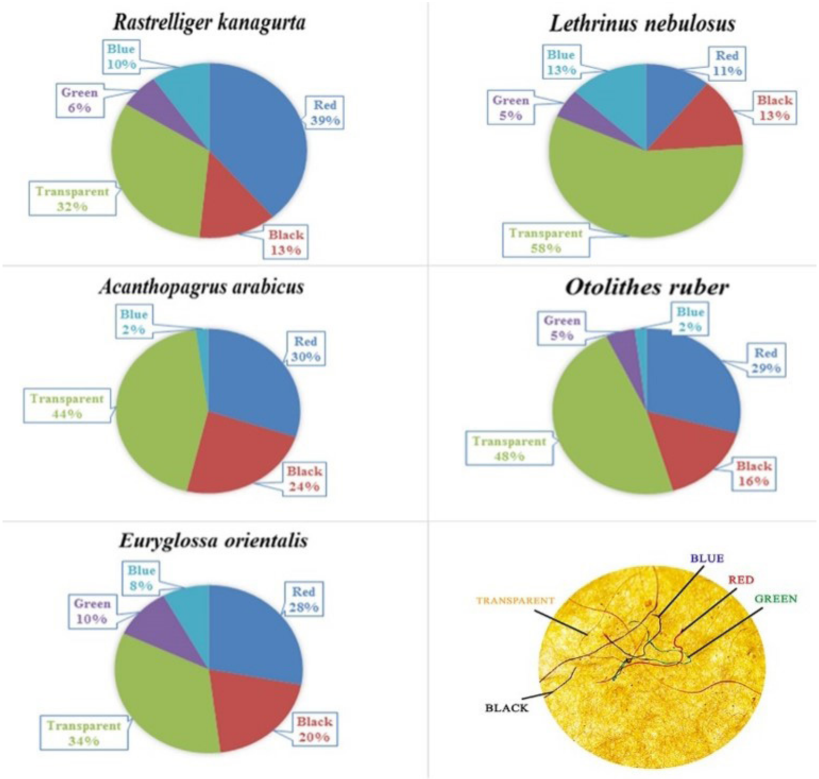

Figure 3. Colour distribution of the microfibers found in fishes collected from the Arabian Sea.

Samples were filtered through three sieves (300, 150, and 50 µm). The result shows that MPs of all three sizes were highest in demersal fishes (*Otolithes ruber* and *Euryglossa orientalis*) except *Lethrinus nebulosus* which had a moderate number of MPs of these sizes along with pelagic-neritic *Acanthopagrus arabicus*. The lowest MPs according to these sizes were recorded in pelagic *Rastrelliger kanagurta* as shown in Figure 4.

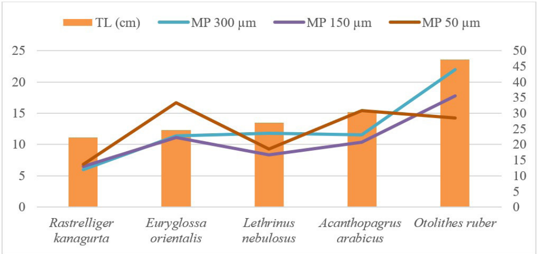

Figure 4. Size-wise distribution of microplastics identified in fishes with different length ranges.

3.3. Validation of microplastics using FTIR

FTIR analysis validated the presence of polyethylene (PE) and polypropylene (PP) in the fish guts. These polymers were identified by their characteristic wave numbers. Figure 5a shows the characteristics absorbance bands for PE which are located at: 2920 cm^{-1}, 2842 cm^{-1}, 1450 cm^{-1}, and 762 cm^{-1}. The peaks at 2920 cm^{-1}, 1450 cm^{-1}, and 762 cm^{-1} are used for identification of PE. Figure 5b shows characteristics absorbance bands for PP which are located at: 2842 cm^{-1}, 1450 cm^{-1}, 1377 cm^{-1}, 1164 cm^{-1}, 974 cm^{-1}, and 840 cm^{-1}. The peaks at 2842 cm^{-1}, 1450 cm^{-1}, 1376 cm^{-1}, and 840 cm^{-1} are used for identification of PP. There were peaks identified as Polyethylene terephthalate (PET) but with noises hence, not considered in present study.

4. Discussion

In this study, microplastics of different shapes and sizes were found in fish collected from the Arabian Sea. Microfibers were the most dominant type of plastic found while beads and films were approximately the same. These results are similar to the studies from the Arabian Sea (Kripa et al., 2014; Kumar et al., 2018). However, a preliminary study from Pakistan showed different results with the highest number of MPs recorded as film particles in marine organisms (Akhter and Panhwar, 2022). We observed variations in types of MPs, MP/fish individual, and MP/g of the fish weight in fish individuals inhabiting different habitats and feeding habits. Similar to the findings of Vendel et al. (2017), there was no significant relationship noticed between the mean values of MPs and the length of the fish. Moreover, MPs 300, 150, and 50 µm sizes were obtained significantly different in numbers among pelagic and demersal fish.

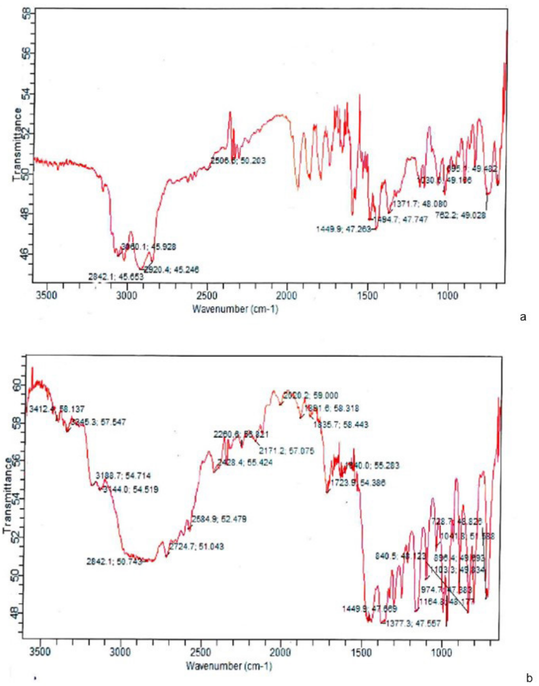

Figure 5. (a) FTIR spectra of polyethylene (PE) found in fish guts; (b) FTIR spectra of polypropylene (PP) found in fish guts.

The separation of plastic particles from samples requires the complete removal of biogenic organic substances. To obtain this, many researchers are using chemical digestion with acids, bases, and oxidizing agents. The optimal digestion method removes the biogenic organic matter as much as possible without affecting the target particles of synthetic polymers. In this study, KOH with different concentrations and exposure times along with oxidizing agent hydrogen peroxide was tested and results showed they were highly efficient in obtaining optimal digestion of the samples (Nuelle et al., 2014; Cole et al., 2014; Karami et al., 2017).

Moreover, without FTIR analysis that provides the plastic polymer identification, the source of microplastics in the sample is hard to point out. Our results of the FTIR spectrum confirmed the presence of polyethylene (PE) and polypropylene (PP) in the guts of fish. The majority of the polymers were identified as PE and PP by their characteristic wave numbers. These results are similar to the findings of Lusher et al. (2013), Rochman et al. (2015), and Kumar et al. (2018). PP was determined with the help of the characteristic wave number in regions of 3000-2840 cm^{-1}, 1459 cm^{-1}, 1376 cm^{-1}, 1167 cm^{-1}, 998 cm^{-1}, 973 cm^{-1} and 840 cm^{-1} while PE was determined with the help of characteristic wave number in regions of 3000-2840 cm^{-1}, 1463 cm^{-1}, and 725 cm^{-1} (Verleye et al., 2001).

Microplastics becoming a part of the marine environment with the increasing demand for plastics worldwide. Many studies have been undertaken globally on occurrence, types, sources, etc. in marine organisms such as molluscs (Li et al., 2015, 2018; Abidli et al., 2019), arthropods (Devriese et al., 2015; Akhter and Panhwar, 2022), echinoderms (De-La-Torre et al., 2020; Sevillano-González et al., 2022), and fishes (Lusher et al., 2013; Saji Kumar et al., 2013; Tanaka and Takada, 2016; Kumar et al., 2018). In Pakistan, reported plastic pollution required attention but the actual unreported condition is alarming. No proper waste management is a key factor. Unsorted and untreated sewerage dumping in the sea worsens the marine habitat and also adds to the level of pollution specifically plastic pollution.

PE and PP polymers are lighter than sea water and hence float on the surface and can easily be ingested by the organisms reaching the food chain. These polymers are used in packaging film, shopping bags, bottles, toys, houseware, juice containers, milk containers, crates, plastic packaging, fibers, and textiles. After use, becomes part of the waste for which no proper waste management system is functional in Karachi. It either enters the marine environment through direct littering or as part of sewerage dumping. We suggest that collective actions are required by governmental and non-governmental organizations to educate and spread awareness of plastic pollution or else the current situation may worsen in the future for marine life and consumers of seafood. Plastic use should be discouraged at all levels and a functional waste management system for disposing of the waste is in dire need.

5. Conclusion

Present study provided microplastics accumulation in the fish gut with polymer characterization from Karachi coastal area of the Arabian Sea. Five types of microplastics were identified in the size range of 300, 150 and 50 µm. Microfibers were the most dominant type of plastic found in almost all individuals. Furthermore, the highest microplastics accumulation was observed in demersal fish while lowest in pelagic. Optimal digestion was achieved by the use of KOH with oxidizing agent hydrogen peroxide in protocol 3 and 4. FTIR analysis validated the presence of polyethylene and polypropylene in the fish guts. This study can be useful for the local environmental restoration agencies and policy makers. We strongly recommend that implementation of a functional waste management system is required to reduce plastic pollution entering into sea.

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