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Distinguishing Microplastics from Microplastic-like particles in the Marine Fish from Qatar

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Abstract

Microplastic (MP) pollution poses a significant threat to marine ecosystems. This study assessed MP accumulation in the gastrointestinal tracts of 170 individuals from four commercially important fish species in Qatar along with their level of risk. MP-like particles were extracted via chemical digestion and density separation, and analysed using stereomicroscopy. Fibers were the dominant MP shape, with blue being the most common colour. The polymer composition of MP-like particles was further analysed using μ-Raman spectroscopy, which confirmed only 7 particles (4.12% of the total 170 fish samples) as MPs (>70% match with polymer library databases). Polymer analysis confirmed the presence of polyethylene (PE) and polypropylene (PP) in the fish digestive tracts. The mean abundance was 0.070 ± 0.090 MPs/g of fish gut, a level relatively low compared to other regions of the Gulf and globally. The herbivorous *Siganus canaliculatus* ingested more MPs than the three carnivorous species, but this difference was not statistically significant ($p > 0.05$).

Keywords: Microplastics, Fish gastrointestinal tracts, Raman spectroscopy, Risk assessment, Arabian Gulf

1. Introduction

Due to rapid increase in industrial activities such as desalination, petrochemical, and fertilizer production, the global annual plastic production has reached 400 million metric tons in 2022[1]. The rapidly growing plastic production and ongoing dependency on fossil fuels have led to significant environmental challenges and health risks[2]. Global plastic production is projected to reach 33 billion metric tons by 2050[3,4]. The term 'microplastics' was first coined by Thompson[5] to describe small size (< 5 mm) plastic particles. More than 92% of the plastics accumulated in the marine ecosystem are microplastics (MPs)[4].

MPs enter the marine environment as primary particles (e.g., industrial pellets, microbeads, or fibers from synthetic textile laundering), or indirectly as secondary fragments from the breakdown of larger plastics[6-9], due to physical (e.g., weathering, wind, water currents or tide abrasion), chemical (e.g., adsorption of organic pollutants or heavy metals) and biological processes (e.g., biofilm formation)[10,11]. In the ocean, they are transported over long distances, and either remain suspended in the water column or accumulate in sediments[12,13].

The hydrophobic nature of MPs enables them to carry toxic pollutants, chemical additives, persistent organic pollutants, heavy metals and pathogens, and thereby increasing their potential toxicity[9,14,15]. The presence of MPs in the ocean is a major concern due to their ubiquity, durability, high chemical stability, persistence, toxic nature, malleability, recyclability, insolubility in water, high bioavailability, non-biodegradability and ability to accumulate in organisms' bodies[14-16].

Due to their small size, MPs can be ingested as prey by a wide range of marine organisms, including fish[16-19], shrimp[20,21], mussels[19,22], turtles[10,23], seabird[10,24] and even zooplankton[25,26]. This ingestion leads to bioaccumulation in their organs and further transferred to higher trophic levels through food chain[27,28], which have detrimental effects such as metabolic disruption[27], leading to decreased fitness and nutritional capacity[14]. In the case of fish, it can cause several adverse effects such as behavioural changes, physical and gastrointestinal damage, intestinal obstruction, metabolic disruption, endocrine system disruption, reduced growth and energy stores, cytotoxicity and even death[14,16,18]. Consequently, this contributes to biodiversity loss, disturbs ecosystem dynamics and threatens the stability of food security[30].

Specifically, the accumulation of MPs in the gastrointestinal tracts (GITs) of fish may have adverse effects on their digestive systems, nutrient absorption and overall health. Studies by[31] and[32] have shown that the presence of MPs in the GITs of fish can cause inflammation, altered intestinal morphology, blockages in the digestive system, and disrupt metabolism and immune function.

In Qatar, fish is a very important renewable resource, with a self-sufficiency rate of 74% in fish products and an average annual consumption of 22.3 kg per capita[33]. Despite this, and increasing global concerns, limited research has been conducted on MP ingestion by commercially important fish species in Qatari waters. Therefore, in the present study, we examined the GITs (which are the organs most commonly accumulating MPs) of 170 fish samples from four species. The fish species used for this study are common commercially available species, purchased from the local fish market/landing centre. We found significant accumulation of MPs in these fish, which will allow researchers to assess the extent of environmental pollution and infer potential pathways through which MPs affect higher trophic levels, including humans. This study aims to assess the presence, abundance and characteristics of MPs in the GITs of these species, which have different feeding habits. The current study is a continuation of preliminary characterization of MPs in 40 fish samples[34], in which a total of 170 fish samples were analysed, and the MPs were extracted, characterized, and confirmed using microscopic and µ-Raman techniques.

2. Materials and Methods

2.1. Study Area

The Arabian/Persian Gulf (hereafter referred to as the Gulf) is a semi-closed extension of the Arabian Sea that extends from the western edge of the Arabian Sea between Iran and the Arabian Peninsula (Figure 1a). The Gulf has a length of 1,000 km and a width of 338 km[35], and it is connected to the Gulf of Oman in the east by the Strait of Hormuz . The Gulf has an average depth of 36 m and is surrounded by subtropical arid coastal areas that support highly productive coastal habitats[36,37]. It is one of the world's warmest and saltiest regions, characterized by high evaporation rates, high temperatures, and hypersaline water.

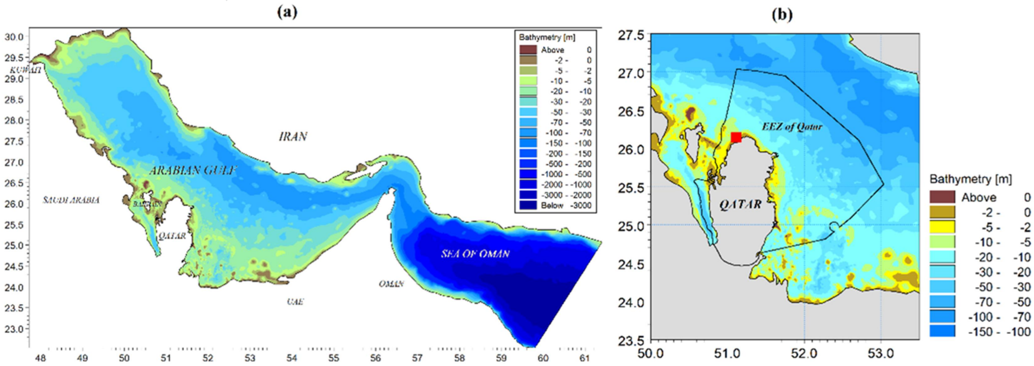

Figure 1. (a) Bathymetry of the Arabian Gulf and (b) EEZ of Qatar. The map was generated by spatially interpolating the digitized bathymetry data using MIKE Zero Tool Box, User Guide. DHI, Denmark, 2017.

Over the Arabian Peninsula, Shamal winds are predominant during winter and early summer, with higher intensity in winter compared to summer, transporting cold and dry air that forms highly saline and cold water in the Gulf[38,39]. The northerly Shamal winds are dominant from November to March (winter Shamal) and from early June to mid-July (summer Shamal). These Shamal winds generate very large shamal swells, which transport large quantities of MPs[40,41].

Qatar is a peninsula (Figure 1b) located on the mid-western coastline of the Arabian Gulf between 24–26°N and 50°30' –51°31'E[8]. Waters surrounding Qatar are shallow, with an average surface temperature of 12°C in winter and around 35°C in summer. The surface salinity ranges between 39 and 41, with elevated values in the bays[42]. The tidal range along the Qatar coast is approximately 2.0 m at Khor Al-Adaid in the southeast[43] and 1.5 m at Fuwairit in the northeast[44].

The Exclusive Economic Zone (EEZ) of Qatar represents 15% of the Gulf and extends over approximately 32,000 km², with depths ranging up to 60 m[45,46]. Qatar's EEZ supports a wide range of ecosystems and provides important ecological, economic, and social benefits to local communities, especially marine fisheries. It supports various marine habitats, including coral reefs, seagrass beds, mangrove swamps, and open ocean areas. These habitats provide essential commercial fisheries resources that are major components of the human diet, nursery grounds for marine organisms, and habitats for many endangered species[47,48]. The EEZ is subject to various environmental pressures such as dredging, reclamation, heavy industrialisation, oil and gas exploitation and marine transportation[49].

2.2. Details of Species

This study investigated four commercially important and commonly consumed fish species in Qatar: Hamour (*Epinephelus coioides*), Safi (*Siganus canaliculatus*), Sheam (*Acanthopagrus* *latus*) and Sheri (*Lethrinus nebulosus*). A total of 170 fish specimens were analysed for MPs. *Epinephelus coioides,* the orange-spotted grouper locally known as Hamour, is the most preferred fish species consumed in Qatar[33,50]. It is a solitary, benthic predator inhabiting in turbid muddy substrates at depths of 1-100 m. This grouper species commonly feeds on shrimps, crabs, and small fishes. *E. coioides* can grow to a maximum length of 120 cm, a maximum weight of 15 kg with a reported lifespan of up to 22 years[51].

Lethrinus nebulosus, the Spangled Emperor (Sheri), is the second most popular fish consumed in Qatar[33]. It is found in various coastal habitats to depths of 75 m, and feeding on small crabs, echinoderms, worms, molluscs, and large zooplankton organisms.

Siganus canaliculatus, the White-spotted spinefoot (Safi), is a herbivorous species that can grow up to 30 cm in length, and inhabiting inshore algal reefs, rocky areas, and coral reefs, where it feeds on benthic algae and seaweed.

Acanthopagrus latus, the Yellowfin seabream (Sheam), distributed in the Indo-West Pacific region, and is characterized by yellow pelvic, anal, and caudal fins[52]. The populations of *Acanthopagrus* (seabreams, also known as porgies) inhabit warm, shallow coastal waters. It is carnivore, feeding on benthic invertebrates such as crustaceans, mollusks and small fish.

2.3. Sample Collection and Preparation

The fish used in this study (*Epinephelus coioides*, *Siganus canaliculatus*), *Acanthopagrus latus*, *Lethrinus nebulosus*) are common commercial available species in Qatar. All specimens were purchased the local fish market/landing centre (Al Ruwais Port) in the northern Qatar. The samples were kept in a closed ice box and transported immediately to the laboratory for processing. In the lab, each fish was thawed and washed with distilled water. The specimens were then measured and weighed using a fish measuring board and a digital scale. The gastrointestinal tracts (GITs) of the fish (including the intestines and stomach) were dissected and extracted from the top of the oesophagus using sterilized scalpels, scissors, and forceps. The extracted GITs were transferred to labelled Petri dishes and stored in the freezer at -80°C until analysis.

As the study utilized GITs from legally caught, dead specimens obtained from a commercial source, and did not involve the sacrifice of animals for research purposes, specific ethical approval for animal experimentation was not required under relevant Qatari regulations and institutional policies. This approach complies with the IUCN Policy Statement on Research Involving Species at Risk of Extinction, as the species are not endangered or at risk.

2.4. Extraction and Filtration of MPs

The GIT samples were processed using slightly modified and established chemical digestion and density separation methods[53-55]. After thawing and rinsing, the samples were weighed and transferred into a series of 500 mL glass beakers. A 10% potassium hydroxide (KOH) solution was added at 1:10 (w/v) ratio. The beakers were covered with aluminium foil and incubated at 60°C for 48-72 hours to ensure complete digestion.

After the digestion, a density separation method was performed by adding 10% sodium iodide (NaI) solution, followed by 10 mL of hydrogen peroxide (H_2O_2, Honeywell 30%) to remove residual organic matter. The solutions were stirred well and left overnight at room temperature. Finally, the supernatant was directly vacuum-filtered through nitrocellulose membrane filters (47 mm diameter, 0.45 µm pore size). The filter papers were air-dried under a fume hood, placed in covered glass Petri dishes, and stored for microscopic and spectroscopic analyses.

2.5. Characterization of MPs

Filter papers were examined and photographed under a ZEISS SteREO Discovery V12 stereomicroscope with Zen 2.6 software (Blue edition, v2.6, Zeiss, Oberkochen, Germany) at a maximum magnification of 60×. This method allowed for high magnifications, generating high-resolution images and enabling precise measurement of very small features and objects. The MP-like particles were counted, and their physical characteristics were assessed. For each particle, the longest dimension was recorded (its size) using the measurement tool in the Zen software. Particles were categorized by shape as follows: fibers (elongated, thread-like), fragments (irregularly shaped, angular pieces), films (thin, sheet-like layers), and pellets/spheres (regular, spherical, or cylindrical forms). Colours were documented based on visual observation under the microscope (e.g., white, blue, red, and black).

Subsequently, the filter papers were used for polymer identification using Raman spectroscopy (DXR3; Thermo Fisher Scientific™, USA), with a 532 nm laser wavelength, 40 accumulations, and laser power ranging from 2 to 10 mW, using a 50x microscope objective. The obtained Raman spectra were compared with chemistry and polymer library databases. Polymers with a match score below 70% were not considered reliable for accurate spectral matching.

2.6. Quality Assurance and Quality Control

To protect the experiment against contamination, no plastic equipment was used during the laboratory analysis. Laboratory work surfaces and metallic tools used for fish dissection were cleaned with 70% ethanol and double-distilled water. All solutions (KOH and NaI) were filtered using nitrocellulose filters. All analyses were conducted under a fume hood. Throughout the experiment, cotton lab coats and nitrile gloves were worn, and all beakers and glass Petri dishes were washed with distilled water. To test for MP pollution in the laboratory, distilled water was passed through filter paper using vacuum filtration under the fume hood. A stereomicroscope confirmed that no MPs were observed in any filters from procedural blanks, confirming minimal or no laboratory contamination with MPs.

2.7. Risk Assessment of MPs

The potential risk assessment of MP consumption in fish species was conducted based on previous studies[56-60]. The Polymer Hazard Index (PHI) was calculated using the following equation:

$$PHI = \sum P_n \times S_n$$

Where, P_n is the percentage of the polymer in the sample, and S_n is the hazard score of plastic polymers. According to[60], the hazard scores of PE, PP, PAN and PA were 11, 1, 11521 and 47, respectively.

2.8.Statistical Analysis

RStudio 4.1.1 and Excel software were used to statistically analyse and investigate the difference between different parameters in this study. By applying RStudio, significant differences between the abundance of MP-like particles in fish gut samples were analysed using ANOVA and Tukey’s test. Linear regression was used to assess the relationship between fish biometric body size parameters and abundance of MP-like particles. Confidence levels for the tests were set at 95 % with significant differences recorded at $p < 0.05$.

3. Results and Discussion

3.1. Abundance of MP-like particles in fish GITs

It is not necessary that all the particles extracted in the filter papers using microscopy analysis are MPs. Most of them could be MP-like particles. It is possible that we may mistakenly report MPs-like particles as MPS. Therefore, we need to make distinction between MPs-like particles and MPS. In this work, we have further analysed MPs-like particles using micro-Raman spectroscopy to confirm their plastic nature (section 3.5). A total of 170 fish specimens representing four different species (*Epinephelus coioides*-50, *Siganus canaliculatus*-80, *Acanthopagrus latus*,-30 and *Lethrinus nebulosus-10*), were used for this study. These species belong to two different trophic levels and feeding habits (carnivorous and herbivorous). The mean total length of the fish ranged between 24.9 ± 4.68 cm (*Siganus canaliculatus*) and 54.4 ± 2.70 cm (*Lethrinus nebulosus*), while the mean weights varied from 224 ± 81.6 g (*Siganus canaliculatus*) to 692 ± 400 g (*Epinephelus coioides*). The data for the analysed fish species, including biometric measurements such as mean length (cm) and mean weight (g) are presented in Table 1.

Table 1. Characteristics of different fish species and their biometric measurements (mean length (cm) and mean weight (g) of fish).

Species	Local name	Feeding habit	Number of examined fish	Number of fish with MP-like particles	Mean length (cm)	Mean weight (g)
Epinephelus coioides	Hamour	Carnivorous	50	31	36.3± 5.36	692± 400.0
Siganus canaliculatus	Safi	Herbivorous	80	78	24.9± 4.68	224± 81.6
Acanthopagrus latus	Sheam	Carnivorous	30	26	24.9± 4.77	337± 223.0
Lethrinus nebulosus	Sheri	Carnivorous	10	9	54.4± 2.70	399± 52.9

MP particles and MP-like particles were observed in the filtration of GIT contents, and they exhibited different size, shape and colour. The abundance of MP-like particles in terms of particles per gram of GIT weight varied between 0 and 3.14 particles/g of gut, for a total of 1043 particles counted. The results indicate that 84.7% of the analysed fish samples had ingested MP-like particles. The abundance of MP-like particles in the guts of four fish species ranged from 0 to 3.14 particles/g in *Acanthopagrus latus*, followed by *Siganus canaliculatus* (0 – 2.87 particles/g), *Lethrinus nebulosus* (0 – 2.34 particles/g), and *Epinephelus coioides* (0 – 0.77 particles/g), respectively with an average of 0.74 ± 0.98 particles/g of gut across all fish samples (before μ-Raman spectroscopy confirmation). According to the results, 135 out of 170 fish samples were contaminated with MP-like particles. The abundance of MP-like particles among the four studied species was statistically significant ($p < 0.05$).

The mean abundance (± SD) of observed MP-like particles in fish GITs was as follows: *Siganus canaliculatus* (2.20 ± 0.42 particles/g), *Lethrinus nebulosus* (0.43 ± 0.63 particles/g), *Acanthopagrus latus* (0.26 ± 0.42 particles/g) and *Epinephelus coioides* (0.090 ± 0.19 particles/g), respectively. The amount of MP-like particles counted in the GITs of *Siganus canaliculatus* was higher compared to the other three fish species (before confirming the particles using μ-Raman spectroscopy). Additionally, no plastic particles were detected in the two procedural blank filters.

Table 2. Number and shapes of MP-like particles found in the GITs of fish.

Species	Pellet	Fiber	Fragment	Film	Foam	Total MP-like particles
Epinephelus coioides	22	61	35	5	0	123
Siganus canaliculatus	85	412	151	26	11	685
Acanthopagrus latus	14	80	32	0	3	129
Lethrinus nebulosus	13	56	28	3	6	106
Total	134	609	246	34	20	1043

Table 2 shows that the *Siganus canaliculatus* species ingested maximum MP-like particles (2.20 ± 0.42 particles/g of gut, which represents 35.8% of the total detected MP-like particles). *Lethrinus nebulosus* and *Acanthopagrus latus* ranked second in the consumption of MPs, accounting for 34.3% and 22.1% of the total detected MPs, respectively. The *Epinephelus* *coioides* species showed the lowest consumption of MP-like particles, accounting for 7.70% of the total detected particles. Based on these findings, it is suggested that the *Siganus canaliculatus* species is likely to consume more MP-like particles than the other three fish species, which could be explained by their feeding habits.

3.2. Characterization of MP-like particles

The extracted MP-like particles from the fish GIT samples were classified as follows: (i) shape: pellet (round, three-dimensional spheres), fiber (thin and long strips), fragment (pieces with a certain thickness), film (thin layer of plastic) and foam (collection of tiny bubbles), (ii) colour: blue, red, black, white, green, yellow and orange and (iii) size: 10 - 50 µm, 50 - 100 µm, 100 - 500 µm, 500 - 1000 µm and 1000 - 5000 µm. We studied and classified these characteristics based on microscopic analysis (Figure 2).

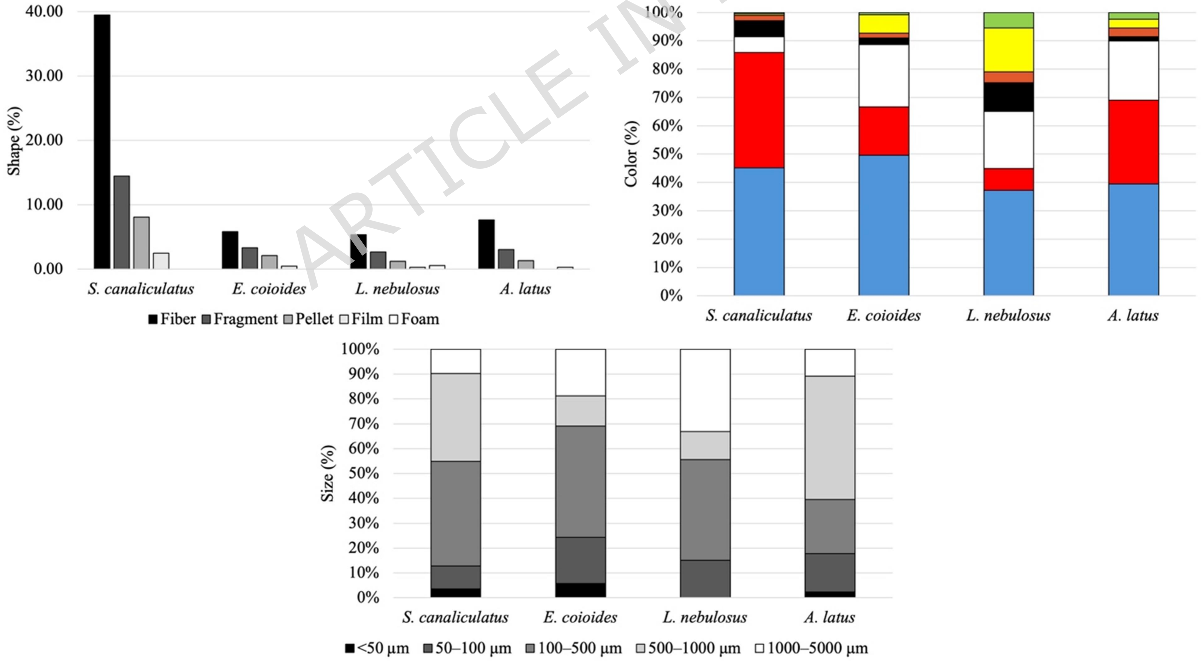

Figure 2. The abundance of MP-like particles in fish samples categorized by shape, size, and colour.

3.2.1. Shape

The 1043 MP-like particles in all fish samples mainly occurred as fibers (58.4% of total particles), fragments (23.6%), followed by pellets (12.8%), films (3.26%) and foams (1.92%), respectively (Figure 3). Fiber particles were the most common shape found in all species, especially in *Siganus canaliculatus*. According to[18], fibers are classified as polyester, which is typically less dense than seawater and most likely to stay in the water column, and misleading the fish as prey. The highest proportion of fibers was observed in the guts of *Siganus canaliculatus* with a percentage of 39.5% of the total detected MP-like particles. Fragments, pellets, and film contents in this species were 14.5%, 8.15% and 2.49%, respectively. This result is consistent with[61], who found that the dominant MP types in *Siganus canaliculatus* were fibers and fragments, with total percentages of 41% and 38%, respectively, however, fragments in our study are lower.

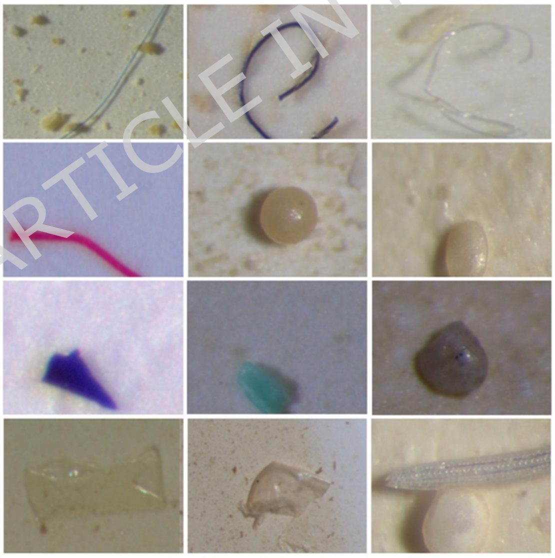

Figure 3. The morphological characteristics of MP-like particles observed under the stereomicroscope

Globally, [22]claim that fibers constitute 90% of the global microplastic concentration, making them the most pervasive marine contaminants. Regionally, the results of this study also match with previous studies conducted in the Arabian Gulf region, the Oman Sea and the Mediterranean Sea and globally in the Caspian Sea, Mondego Estuary, South China, and Pantai Indah Kapuk coast[62-65], where fibers were found to be ubiquitous in the marine environment[66,67]. A possible source of fibers found in the samples might be from the degradation of fishing nets, lines, and ropes as a result of fishing activities[42,68], in addition to atmospheric fibers[69]. However, our results do not agree with[70], who stated that the fragment shape of MPs was the most ingested shape by benthic organisms. Table 3 represents different studies that were carried out in the Gulf to investigate the abundance of MPs in the GITs of fish, showing that fibers were the main shape of MPs detected.

Table 3. Comparison of average MPs abundance in the GITs of commercial fish in the Arabian Gulf.

			Predominant MP particles			
Location	No. of samples	MPs mean abundance (MPs/g fish guts)	Shape	Colour	Size	Reference
Western Arabian Gulf	140	0.057 ± 0.019	Fibers	Blue	1.09 ± 0.05 mm	[16]
Northern coasts of the Persian Gulf	110	2.46 ± 1.46	Fibers	Black	23–75 µm	[14]
Northern coast of Persian Gulf	280	0.33 ± 0.05	Fibers	Black	1000–2000 µm	[71]
Bushehr Province, Persian Gulf	60	2.90 ± 0.82	Fibers	Black	500–1000 µm	[72]
Northwest of Persian Gulf	80	0.147	Fibers	Black	500–1000 µm	[69]
Qatar, Arabian Gulf	**170**	**0.070 ± 0.090**	**Fibers**	**Blue**	**100–500 µm**	**Present study**

3.2.2. Colour

For a more convenient interpretation, MP-like particles were also categorized by colour using a stereomicroscope. Since fish are visual predators, they are mistaken by the colour of MPs due to their resemblance to natural prey[58,73]. The MP-like particles showed a diversity of colours in the samples. Blue was the most dominant colour of MP-like particles observed in this study and had the highest abundance among all the analysed samples, accounting for 45.0%. This is followed by red and white colours, which accounted for 33.4% and 11.3% in all analysed samples, respectively. Other colours (black, yellow, orange and green) accounted for only smaller proportions (5.47%, 2.30%, 1.92% and 0.67%, respectively). In the case of *Siganus canaliculatus*, blue-coloured particles occupied 29.6%, followed by red and white. The colouration of MPs allows a variety of marine organisms to unintentionally ingest the particles as their characteristics are almost identical to their natural prey. The different colours of MPs in the environment increase the potential for MPs ingestion due to their similarity to natural prey as has been observed by many previous researchers[64,74]. In this study, the GITs of *Siganus canaliculatus* showed more blue-coloured particles, which is in agreement with the results of a previous study conducted in Qatar by[45], where the blue colour of MPs was the most common in the Qatari waters. Studies by[67] and[16] also reported that blue was the most prevalent colour among the commercial fish species.

3.2.3. Size

The MP-like particles detected in this study ranged from 10 µm to 5000 µm. In this study, MP-like particles were divided into five groups: 10 - 50 µm, 50 - 100 µm, 100 - 500 µm, 500 - 1000 µm, and 1000 - 5000 µm. The size distribution pattern of MP-like particles in fish guts showed that the highest abundance of detected particles was in the 100 - 500 µm size range. Particles in the 100 - 500 µm size range were the most common in all samples (39.7%), followed by 500 -

1000 µm, 1000 - 5000 µm and 50 - 100 µm, accounting for 31.9%, 13.3% and 11.8%, respectively. Only 3.26% of the total MP-like particles detected in the analysed samples were in the range of 10 - 50 µm in size. The different sizes of MPs could be explained by various biological and physiological degradation of the particles over time[54]. The small size of MP particles gives them the potential to be ingested by a variety of marine organisms. The small size of MPs provides a high surface area, increasing the chances of the adsorption of toxic compounds in the environment[75]. The feeding mechanism of some organisms does not allow for discrimination between target natural prey and suspended MP particles in water, leading to accidental ingestion[76,77]. MP ingestion is mostly correlated with MP concentrations in the aquatic ecosystem[54]. While some organisms intentionally ingest MPs mistaking them for prey, some others selectively feed on organisms whose GITs are already accumulated with MP particles[78]. This is due to their failure to distinguish between their prey and foreign particles.

3.3. Distribution of MPs based on fish size

According to the results, a negative correlation was found between biometric body mass of different species and abundance of MP-like particles in fish guts. Findings showed that the highest accumulation of MP-like particles in the gut was observed in *Acanthopagrus latus* fish, which has a length of 22 cm and a weight of 210 g, in contrast to accumulation of 0 MPs/g gut in most of the *Epinephelus coioides* fish, which had a length of 33 to 37 cm, and a weight of 300 to 600 g. A regression analysis was carried out to indicate the relationship between weight, length, and gut weight with the abundance of MP-like particles, but it showed no significant association between fish size and the MP ingestion. The correlation coefficient between all fish sizes and MP ingestion was < 0.3, indicating a weak negative association between these factors. This suggests that the abundance of ingested MPs was not strongly dependent on the fish weight, length or gut weight ($r = 0.24$; $p > 0.05$ for correlation with fish weight, $r = 0.14$; $p > 0.05$ for correlation with fish length, and $r = 0.17$; $p > 0.05$ for correlation with fish gut weight). The ingested MPs-like particles did not significantly differ among different fish species according to fish size as previously reported in different studies[61,55,79,80]. In addition to biometric body size parameters, many important factors such as feeding habits and habitat, which influence MP ingestion need further study.

3.4. Habitat and feeding habits

Demersal fish generally live and feed on or near the seabed and benthic areas. Many demersal species depend on pelagic production by feeding on vertically migrating nekton or zooplankton living near the bottom. Regarding feeding habits, some fish have a highly selective diet, while others eat whatever is available. Due to their close association with the seabed, demersal fish are considered small-scale indicators of benthic habitat contamination. It is advantageous to study these species since marine sediments have been identified as an important sink and ultimate endpoint for MPs[81]. The demersal/benthic fish are most exposed to MPs and more susceptible to accumulating much higher concentrations of MPs compared to pelagic fish species. This difference may be due to the sinking of MPs in the water column and the accumulation of plastic waste near the seabed[66,75,80,82]. In this study, the selected four fish species are classified as demersal fish as they feed mainly on benthic invertebrates (*Epinephelus coioides*, *Acanthopagrus latus*, and *Lethrinus nebulosus*) and marine plants (*Siganus canaliculatus*). Several global studies have investigated the presence of MPs in fish GITs based on feeding habits to provide a better understanding of MPs intake and ingestion. In this study, *Siganus canaliculatus,* which feeds on plants, ingested more MPs than the other three carnivorous fish, but the correlation between the herbivore feeding habit and the abundance of MPs was not significant ($p > 0.05$). According to[83] study, herbivorous fish are more likely to mistake and ingest MPs present in the water column compared to carnivorous fish. Herbivorous fish feed on seagrasses, algae and aquatic plants and are characterized by an adapted digestive system to efficiently digest plant material in addition to small plastic particles mistaken for food. MPs adhere to the surface of algae and attach to the leaf surfaces of seaweed allow herbivorous fish to ingest MPs accidentally while feeding on plant materials that had been previously exposed to MPs[61,84]. Researchers have investigated the relationship between fish feeding behaviour and MP ingestion, and provided evidence that marine plants may act as a vector for MPs to marine herbivores. For example, similar to our results, several researchers recorded higher rates of MP uptake in herbivorous fish species[61,80,85]. [86]noted that MPs attached to the surface of algae laminae leads to accidental ingestion by herbivorous fish. They further reported that benthic seaweed can retain suspended MPs on its surface[87], making them accessible to herbivores. This concern is reinforced by recent evidence confirming the transfer of MPs from contaminated macroalgae to higher trophic levels, including fish, highlighting a direct pathway for MP ingestion via herbivory[88]. Another study found that the guts of *Siganus canaliculatus* contain a high amount of zooplankton despite the species being primarily herbivorous[89]. It is possible that MPs are ingested by these zooplankton while feeding. Similarly, [90]demonstrated that zooplankton ingest MPs, which can directly affect the zooplankton or indirectly impact species that feed on them. *Siganus canaliculatus* is classified as an important macroalgal grazer[91], primarily feeding on sediment, which serves as a major sink for MPs. [92]found that MP concentrations were higher in sediments within seagrass habitats compared to non-vegetated areas.

Another important factor contributing to the high abundance of MPs in Safi samples may be gut physiology. Herbivorous fish tend to have thicker stomach walls and longer digestive tracts with a large surface area than carnivore fish, allowing them to consume larger amounts of food[93-96]. This results in prolonged retention of food within the digestive system. Consequently, as the retention time for ingested material increases in longer guts, there is greater potential for MPs to accumulate and get detected in the GIT[97]. [98]found that the extended digestive process of *Siganus canaliculatus* enables them to use highly acidic stomach fluids to break down their food. [99-101]reported that the cellulose matrix of plant cell walls promotes binding between algae and plastics through electrostatic attraction to cellulose components. Similarly, [87]noted that plastic particles attached to algal surfaces via electrostatic binding to cellulose may explain the higher accumulation of MPs in the guts of herbivorous fish. However, our results did not align with the findings of previous studies[53,64,102], suggesting alternative interpretations. This could be attributed to the differences in species, sample size, sampling location, habitat, methods used and biological and physiological characteristics. [103]showed that the abundance of MPs was not influenced by trophic transfer and fish biological parameters. Moreover, some researchers suggest that MPs can be swiftly cleansed from organisms, thereby unlikely to affect organisms at higher trophic levels[83].

3.5. Chemical characterization of MPs

3.5.1. Micro-Raman spectroscopy analysis

In order to confirm the plastic nature of the extracted MP-like particles as MPs, the filter papers used for the microscopy analysis were further analysed using micro-Raman spectroscopy. Among the total 1043 MP-like particles detected from 170 fish samples, only 162 particles were confirmed as MPs, and other particles were sorted as MP-like particles. The order of MP ingestion found in the studied fish species is as follows: *Siganus canaliculatus* > *Acanthopagrus latus* > *Epinephelus coioides* > *Lethrinus nebulosus*.

Overall, nine different types of polymers and co-polymers were identified: polypropylene (PP), chlorinated polypropylene (CPP), polypropylene glycol (PPG), polyethylene (PE), polyethylene-co-vinyl acetate (PEVA), polyethylene-co-methacrylic acid (PEMA), polyisobutene (PIB), polyacrylonitrile (PAN), polyamide (PA). Micro Raman spectroscopy showed that 15.5% of the detected MP-like particles were MPs. The confirmed particles were predominantly CPP, which accounted for 70.4 % of the total identified MPs, followed by PE (10.5%), PP (9.26%), PEVA (3.70%), PEMA (1.85%), PIB (1.85%), PA (1.23%), PAN (0.62%), and PPG (0.62%).

In the GITs of *Siganus canaliculatus*, the polymers identified were primarily CPP (71.9 %), followed by PE (14.6 %) and others (PP, PEVA, PPG, PEMA and PA). PIB and PAN polymers were present only in *Lethrinus nebulosus*, while all the *Epinephelus coioides* samples showed the presence of CPP. Fish have the potential to be exposed to, ingest, or absorb these synthetic polymers from their environment. This raises concerns for environmental and health studies, as it may indicate pollution and potential impacts on aquatic life and the food chain.

3.5.2. Analysis with reference spectra from polymer library

All reported polymers were matched with reference spectra from a polymer library, with percentages ranging from 13.2 to 91.4%. According to [104,105], spectral matches with a high-quality index (> 70% match) are considered reliable for polymer identification, while lower matches are disregarded and treated as unidentified particles due to fragmentation and biofouling processes, which affect the degree of spectral alignment. Applying this >70% quality threshold to our study resulted in a revised classification of the particles. Ultimately, µ-Raman spectroscopy confirmed MPs in only 7 of the 162 visually identified particles, that is, 4.32% were verified as synthetic polymers. The representative Raman spectra of MPs identified in this study are shown in Figure 4. Among the confirmed MPs (> 70% match), the most prevalent polymer was PEVA (42.9%), followed by PEMA (28.8%), PE (14.3%) and PP (14.3%). The detailed analysis showed that 85.7% of the confirmed MPs were found in *Siganus canaliculatus* with a mean abundance of 0.18 MPs/g gut and 14.3% in *Acanthopagrus latus* with a mean abundance of 0.10 MPs/g gut. No confirmed MPs were found in *Epinephelus coioides* and *Lethrinus nebulosus*. The abundance of confirmed MP particles in the guts of seven fish samples (4.12% of the total samples) after μ Raman spectroscopy analysis ranged from 0 to 0.18 MPs/g with an average of 0.070 ± 0.09 MP/g across all samples. The abundance of MPs in this study is relatively low compared to levels reported in other regions of the Gulf and in many studies worldwide. One of the reasons could be limitation in the methodology and detailed identification followed in distinguishing MPs from MP-like particles in some of the earlier studies.

The polymer composition of MPs confirmed that the PP and PE are mainly derived from single-use plastic packaging items (land-derived sources), whereas PA is mainly originated from the fishing industry (sea-derived sources). PP and PE were identified as the most commonly found polymers in sediment and water environments and these are ingested by fish. These polymers make up more than 80% of the total market demand and are widely used in the production of disposable products[16,106].

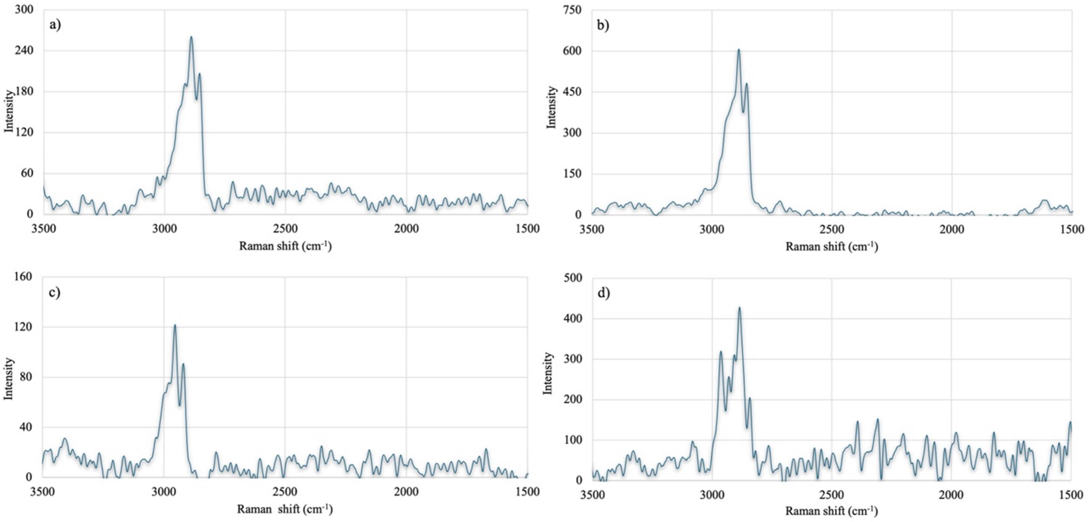

Figure 4. Raman spectra of: a) PEVA, b) PEMA, c) PE and d) PP.

3.6.MPs risk assessment

The polymer hazard index (PHI) is one of the preliminary risk assessment models used to assess the risk of the MPs in different fish species. The PHI can be used to detect the hazard level of polymer pollution caused by MPs, categorizing the risk of MP pollution in samples from level I (minor pollution) to IV (extreme danger).

The species *Siganus canaliculatus* had the highest pollution value (PHI = 66), and was categorized as having a high level of pollution (10 < PHI < 100) due to the presence of large numbers of polymers. This was followed by *Acanthopagrus latus* (PHI = 1), which was categorized as level I risk (PHI < 10), with minor pollution. The values for *Lethrinus nebulosus* (PHI = 0) and *Epinephelus coioides* (PHI = 0) indicated no pollution in these species.

We could not compare PHI values of this study with those of earlier studies of the Arabian Gulf (as shown in Table 3) as no PHI data are available, and in some studies, polymer analysis was also not performed. Although direct comparison within the region are not possible, global studies have reported much higher PHI values[58,72,107,108].

4. Conclusions

The presence of MPs in the marine environment certainly contributes to the pollution of edible fish, although not to dangerous level. When a total of 170 fish samples were analysed, only 4.12% of the examined fish were confirmed to be contaminated with MPs, with an abundance of 0.070 ± 0.090 MP/g fish gut. This indicates a low average value compared to other studies in the Gulf and globally. The polymer composition of MP-like particles was identified using μ-Raman spectroscopy, with only 7 particles confirmed as MPs (showing >70% match with polymer library databases). The main polymers detected in the fish species, *Siganus canaliculatus* and *Acanthopagrus latus,* were PEVA, PEMA, PE and PP. The calculated MP risk assessment using the polymer hazard index suggested different hazard levels in two species, with the highest risk level observed in *Siganus canaliculatus*. Further detailed studies are needed to evaluate the presence of MPs in many species of fish and their consequences in the marine environment, which is a limitation of this study. More efforts are also required to better understand the ecological risks and their implications for human health.

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Author Contributions

S.D: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. A.M.: Writing – original draft, Methodology, Investigation, Formal analysis. F.A.A: Writing – review & editing, Validation, Investigation, Conceptualization. S.V: Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. A.V.M: Writing – review & editing, Validation, Investigation. J.A: Writing – review & editing, Validation, Supervision, Investigation, Conceptualization. P.V: Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. All authors reviewed the manuscript.

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Conflict of Interest

The authors declare no competing interests, and have no relevant financial or non-financial interests to disclose.

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Data availability

YES.

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Data used in this study will be made available on request to the above Contact person, who is Corresponding author of this manuscript.

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