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Occurrence and Characterization of Microplastics in the Digestive System and Gills of Yellowfin Tuna (*Thunnus albacares*) from the Oman Sea

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ABSTRACT

Aims: Yellowfin tuna (*Thunnus albacares*), a commercially and ecologically significant migratory species, is vulnerable to microplastic ingestion because of its open-mouth swimming and extensive foraging range. This study aimed to provide the first evidence of microplastic presence and characteristics in the digestive tract and gills of this species from the understudied Oman Sea.

Materials & Methods: Twenty specimens were collected from artisanal fisheries in the Oman Sea near Chabahar. Digestive and gill tissues were digested using a modified KOH protocol optimized for wet samples, followed by density separation, filtration, and stereomicroscopy. FTIR characterized selected particles.

Findings: Microplastics were present in 80% of digestive tracts (1–25 particles per fish) and 75% of gills (1–4 particles per fish), with fibers being the most common (85% in the gut, 81% in the gills), mostly transparent or blue. FTIR confirmed nylon and polyethylene.

Conclusion: This initial report highlights high microplastic exposure in yellowfin tuna from the Oman Sea, with greater accumulation in the gut, underscoring the need for ongoing monitoring in this area.

Keywords: Marine Pollution; Micro-Plastics; Yellowfin Tuna; Oman Sea; Water Pollutant.

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Introduction

Tuna species are among the most economically and ecologically significant pelagic fishes worldwide. While the term "tuna" broadly refers to several scombrid species, it is most often associated with large, high-value members of the genus Thunnus, such as yellowfin and bigeye tuna, which inhabit tropical and subtropical oceans [1]. These species possess unique anatomical, biochemical, and bodily adaptations that support an energy-intensive lifestyle characterized by continuous swimming, high metabolic rates, and increased oxygen demand [2]. Due to unpredictable prey supply and the need to forage over vast oceanic areas, tuna species have developed feeding strategies that maximize food intake per feeding event to offset their high energy expenditure [3]. However, rising commercial demand has increased fishing pressure, leading to a significant decline in tuna populations across many marine regions [1]. Yellowfin tuna (*Thunnus albacares*) is a highly migratory predator found broadly in tropical waters, including saline and brackish environments, typically at temperatures between 15 and 31 °C. This species swims quickly with its mouth open to meet its high oxygen needs, which may also increase its exposure to suspended particles and contaminants in the water column [4]. These ecological and physiological traits make yellowfin tuna particularly vulnerable to ingesting human-made pollutants, including microplastics. Among emerging marine pollutants, microplastics have gained global attention due to their widespread presence and persistence in aquatic environments. Plastics are synthetic polymer materials used extensively across many sectors, including packaging, transportation, healthcare, agriculture, and fisheries [5]. Common polymer types like polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyurethane (PU), and polyamide (PA) dominate global plastic production [6]. They break down through physical, chemical, and biological processes such as photolysis, hydrolysis, thermal degradation, and mechanical wear, causing larger plastic debris to fragment into smaller particles called microplastics [7]. Microplastics are defined as plastic fragments smaller than 5 mm and are categorized as either primary microplastics, manufactured at microscopic sizes, or secondary microplastics, resulting from the breakdown of larger plastics [8-9]. They come in various shapes, including fibers, fragments, films, and pellets, and their environmental distribution depends heavily on polymer density. Low-density plastics like PE and PP tend to remain afloat or be suspended in surface waters, while higher-density plastics are more likely to sink and accumulate in sediments [10]. The rise in global plastic production, from about 0.5 million tons in 1960 to nearly 300 million tons in 2013, has increased the amount of plastic debris in marine ecosystems [11]. It is projected that global plastic production will reach around 440–450 million tons by 2025, with an estimated 0.3 million tons of microplastics entering the oceans in 2019. Plastics in the environment are forecast to reach around 12 billion tons by 2025, with global production possibly approaching 1 billion tons by 2060 and micro- and nanoplastics entering the oceans at about 0.8 million tons annually, nearly tripling the 2019 level [12]. As a result, microplastics are found in many marine habitats from surface waters and

coastal zones to deep-sea environments and polar regions [13-14]. Besides their physical presence, microplastics pose ecological risks because they can absorb and adsorb a wide range of chemical pollutants. Processes such as weathering, UV radiation, and biofilm formation increase their surface area and reactivity, thereby enhancing their ability to interact with contaminants [15-16]. Pollutants linked to microplastics include hydrophobic organic compounds like polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), as well as hydrophilic substances such as perfluoroalkyl acids (PFAAs), pharmaceuticals, and heavy metals [17]. Factors like particle size, color, polymer type, age, and environmental conditions (e.g., salinity and pH) further influence how pollutants interact with microplastics [18]. The ingestion of contaminated microplastics by marine organisms across different trophic levels has been shown to disrupt metabolic functions and cause adverse biological effects [19]. Consequently, the transfer of microplastics through marine food webs raises concerns about seafood safety and potential impacts on human health [20-21]. Microplastics enter marine environments via various pathways, including wastewater discharge, urban and industrial runoff, agricultural runoff, atmospheric deposition, maritime activities, fisheries, aquaculture, and tourism [22-26]. Once in the ocean, these particles undergo complex transport and redistribution driven by ocean currents and influenced by particle characteristics such as size, shape, and density [27]. The fragmentation of microplastics into nanoplastics increases their bioavailability and potential for biological uptake, affecting feeding behavior, digestion, and physiology in marine organisms [28-31].

Despite the growing body of research on microplastic pollution, significant knowledge gaps remain regarding the occurrence and distribution of microplastics in commercially important pelagic fish species, particularly in understudied regions such as the Oman Sea. Limited information exists on the presence of microplastics in various tissues of yellowfin tuna, including both the gastrointestinal tract and respiratory organs, which serve as distinct exposure pathways. Therefore, this study aims to investigate the presence and characteristics of microplastics in the gastrointestinal tract and gills of yellowfin tuna (*Thunnus albacares*) collected from the Oman Sea, providing baseline data to enhance understanding of microplastic contamination in this ecologically and economically vital species.

Materials & Methods

Sample Collection

With the coordination of the Sistan and Baluchestan Province Industrial Towns Company, a total of 20 fish samples were randomly collected from fishermen operating in the Oman Sea in Chabahar and immediately transported to the university laboratory. Afterward, the total length of each fish (from the tip of the snout to the end of the caudal fin) was measured using a biological measuring board (100 cm long, with an accuracy of ± 1 mm), and their total weight was recorded with a digital scale accurate to ± 10 g (Table 1). Subsequently, the digestive system of each fish, from the esophagus to the anus, was carefully removed, and the gills were completely extracted. After separation, the muscle tissues were sent to a tuna canning factory located in the Chabahar industrial town. The samples were numbered for later processing, wrapped in aluminum foil, and stored in a freezer at

−20°C. Sampling took place in early February 2023, and all laboratory analyses began immediately after sampling.

Table 1) Specifications of Examined Samples.

Sample Number	Total Length (cm)	Fish Weight (kg)	Wet Weight of the Digestive System (g)	Wet Weight of the Gills (g)
1	79.5	8.02	418.48	178.3
2	68.3	6.55	404.71	241.63
3	77.2	9.1	380.15	401.75
4	69.1	6.11	219.66	284.89
5	74.7	6.95	283.03	313.83
6	77.3	7.53	319.89	345
7	67.5	6.05	205.3	225.22
8	70.1	6.57	289.44	277.83
9	66.8	5.17	326.02	277.2
10	65.4	4.53	168.25	213.12
11	74.3	6.9	288.22	300.1
12	67.9	5.59	220.98	277.75
13	64.6	4.07	295.73	266.44
14	68.2	5.5	187.36	233.96
15	64.9	4.53	201.45	221.85
16	66.8	5.45	185.75	228.77
17	66.3	4.58	309.57	342.9
18	66.5	4.71	219.1	195.16
19	64.1	4.82	185.69	209.8
20	82	6.43	280.34	237.96

Sample Preparation

For analysis, each sample was first brought to room temperature to ensure complete thawing. After thawing, the outer surface of the digestive system was thoroughly rinsed with double-filtered distilled water to remove any external contaminants. The wet weight of the samples was then measured and recorded using a digital scale (Model AND EK-610i, accuracy 0.01 g).

Microplastic Extraction from the Digestive System and Gills

The digestive system was placed on a metal tray for dissection and visual inspection to determine whether it was full or empty. Initially, the presence or absence of plastic particles was examined visually. Next, to digest the biological tissue, the digestive system and gills were placed in separate beakers, and 10% Potassium Hydroxide (KOH) solution (Merck, Germany) was added to each beaker. In several common protocols, the recommended amount of potassium hydroxide for digesting dry tissue is 10 $mL.g^{-1}$ of tissue [1-3]; however, preliminary tests showed that this approach was insufficiently effective for the samples in this study. Therefore, to optimize digestion and given the relatively large sample size, the amount of potassium hydroxide was adjusted based on each sample's wet weight. Ultimately, 3 g of KOH per 1 g of wet sample was found to be optimal, resulting in complete digestion without damaging the plastic particles. After adding the KOH, the samples were placed in an oven at 40°C to enhance reactivity. After 48 to 72 hours, the biological tissue was fully digested. The solution was then filtered through Whatman filter paper grade 42 to remove any residual material. To assist in separating plastics from other materials, an ultrasonic device was used, and $ZnCl_2$ solution (produced by Merck, Germany) was added to increase density and help the plastics float. The filter paper was placed in a Petri dish, and a 1.52 $g.cm^{-3}$ $ZnCl_2$ solution was added. The dish was then placed in the ultrasonic device for 10 to 15 minutes. Afterward, the filter paper was removed and placed on a magnetic stirrer for 30 minutes to detach any plastics adhering to the residues.

Due to density differences, plastics floated on the surface. The upper solution was collected, transferred to a test tube, and

centrifuged at 5000 rpm for 5 minutes. After centrifugation, the upper layer was filtered through a 0.22 µm pore-size membrane filter (MCE Membrane Filter) using a vacuum pump. The filters were then placed in 6 mm Petri dishes to dry and prepare for microscopic analysis. To count, measure, and assess the color and shape of microplastics, the filters were examined under a stereomicroscope (Nikon SMZ1000, model C-DS, Japan) equipped with a Dino-Lite digital camera and Dino Capture software. Images of each microplastic were taken, and their features were recorded. The dimensions (in mm) of each particle were measured with ImageJ software.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

To analyze the infrared spectra of selected samples, FTIR spectroscopy (Thermo Nicolet, model AVATAR 370 FT-IR, USA) was performed at a resolution of 4 cm^{-1} over the range 4000–400 cm^{-1}, and the spectra were recorded.

Quality Assurance and Control

All equipment was sterilized with 10% nitric acid, rinsed with double-filtered distilled water, and dried in an oven at 60°C. They were then covered with aluminum foil. The experiments took place in a clean, isolated environment. Metal and glass tools were used to prevent contamination. Filter paper was examined under a microscope before use, and no microplastic contamination was detected. All steps in the materials and methods adhered to established protocols [1, 29-31].

Findings

Abundance

The investigation showed that microplastics were found in the gastrointestinal tract of 85% of the samples (Figure 1) and in the gill tissues of 75% of the samples (Figure 2).

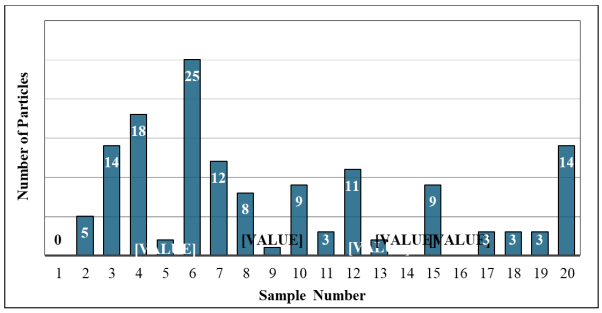

Figure 1) Count of plastic particles in the gastrointestinal tract of the samples.

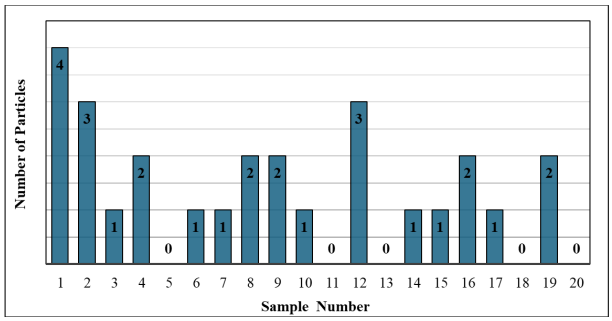

Figure 2) Count of plastic particles in the gills of the samples.

Overall, microplastics were found in 100% of the analyzed fish specimens, with a range of 1 to 26 particles per individual. Recent global studies show similar results, some of which are referenced below [5]. A study conducted in Bangladesh in 2023, analyzing 20 marine species, reported the presence of plastic in the gastrointestinal tracts of all samples (100%), with varying counts per fish. This was linked to wastewater discharge into marine environments and water currents transporting plastic debris. These findings match the results of the current study [6]. In Spain, a 2023 study on *Engraulis encrasicolus* and *Sardina pilchardus* found microplastics in all lab-analyzed specimens, indicating a 100% prevalence. The study attributed this to the widespread distribution of plastic pollution across aquatic ecosystems

and the increasing introduction of these contaminants, especially into marine habitats through various pathways. The fish contained between 11.5 and 94.8 microplastic particles each. Similarly, the current study confirmed a 100% occurrence rate, with counts ranging from 1 to 26 particles per specimen [7]. On the southern coast of Java, Indonesia, research on 10 skipjack tuna (*Euthynnus affinis*) showed microplastics in the gastrointestinal tracts of all samples, consistent with this study's findings. This was linked to the species' migratory nature, the high level of plastic pollution, and the fish's predatory habits. Additional studies further support these factors [8]. One investigation into microplastic pollution in 15 commercially important dolphinfish (*Coryphaena hippurus L.*) caught in the eastern Pacific Ocean found 1,741 suspected plastic particles in their gills, gastrointestinal tracts, and muscle tissues. Of these, only 139 particles were confirmed to be microplastics using microscopy and micro-Raman spectroscopy. All 15 fish (100%) contained microplastics, with an average of 9.3 particles per fish. This was attributed to their large size, older age, and widespread oceanic plastic pollution. These results closely resemble those of the present study [9]. Findings from the southwestern Atlantic Ocean reported a 68% prevalence of microplastics in the gastrointestinal tracts of lanternfish (*Myctophidae*), collected from depths up to 1,000 meters. Despite most plastics being less dense than seawater, the presence of microplastics at such depths suggests they either adhered to sediments and sank or were made of denser materials. Compared to this study, the reported abundance is lower. Microplastics have also been identified in South African marine waters. Overall, this study's findings align with reports from various regions worldwide, highlighting the widespread contamination of marine ecosystems by microplastics.

Morphology

After examining the gastrointestinal tracts and gills of 20 samples, 142 suspicious particles were identified in the gastrointestinal tracts (Figure 1). Among these, 121 were fibers, 15 were films (thin layers), and 6 had irregular shapes. In the gills, only 27 suspicious particles were found (Figure 2), of which 22 were fibers, and 5 were films (Figures 3, 4, and 5).

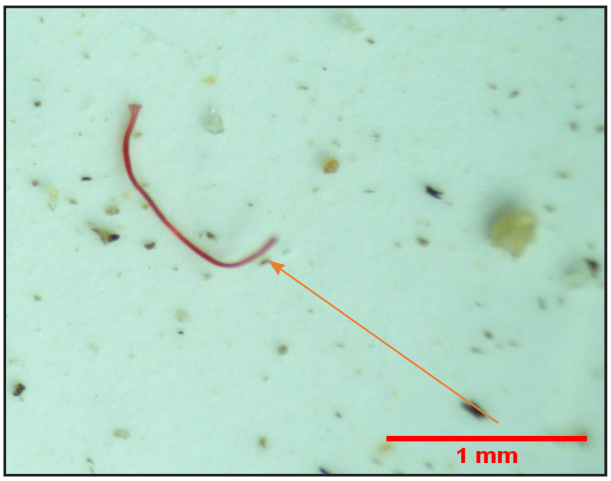

Figure 3) An image of a fragment viewed under the microscope.

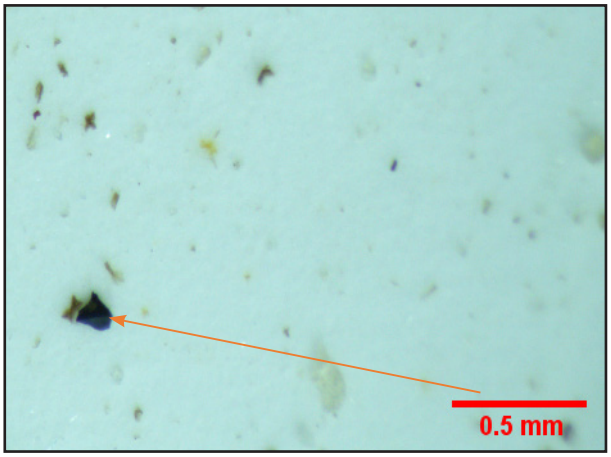

Figure 4) An image of a fiber viewed under the microscope.

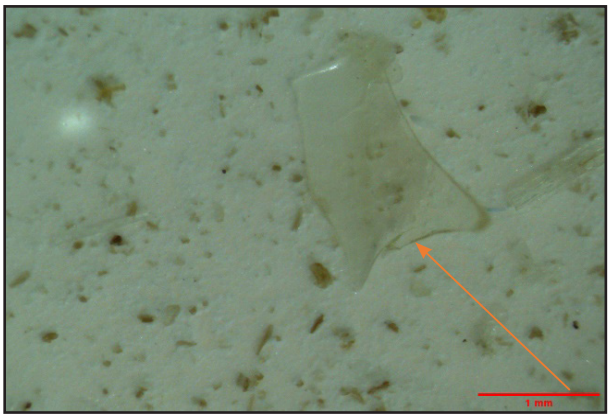

Figure 5) An image of a film viewed under a microscope.

Figures 3, 4, and 5 show images corresponding to the film, fiber, and irregular-shape categories. Comparing these results with various studies from around the world confirms the similarity of the findings. In a study conducted on skipjack tuna purchased from 5 randomly selected fish markets along the Indonesian coast, the distribution of particle types was as follows: 84% fibers, 11% films, and 5% irregular shapes. As previously mentioned, the predominance of fibers in the present study is evident [32]. In another study, the types of microplastics identified in the gastrointestinal tracts of two commercially important fish species from 6 stations in the southern Caspian Sea were reported. The majority of findings were fibers (50%), followed by fragments (30%), with the remaining 20% attributed to other forms [33]. The laboratory results of Dr. Najji and his colleagues' study on the gastrointestinal tracts of *Chelon aurata* and *Rutilus kutum* in the Persian Gulf indicate that fibers were the most abundant, accounting for 83%, followed by films at 11%, and irregular shapes at 6% [34]. In another study, 18 species from the southern coast of China were evaluated, and all the plastics found in the samples (100%) were fibers. In the studies we conducted during and prior to this project, it was very rare for all suspicious particles, even in 18 different species, to be of the same form [35]. Recent studies conducted in Turkey show that analysis of several economically important fish species revealed that nearly 80% of the suspected microplastic materials found were fiber [36]. A research study conducted in the Yangtze River estuary and the coastal area of the East China Sea in 2014 showed that fibers were the dominant morphotype in both regions, accounting for up to 79% and 83% of the microplastics collected at the two sites, respectively [37]. According to researchers, one of the areas that is heavily impacted by human activities is the enclosed ecosystem of Jiaozhou Bay in China. The most common type of microplastic found in this study was fibers, with black or blue fibers being the predominant findings, which are the main characteristic of the microplastics present in the collected samples [38]. In most of the cases examined in various studies, fibers hold the top position, and one of the main reasons for this is as mentioned by [39]. It was noted that fibers from synthetic textiles are being released into the sea.

Color of the Detected Microplastics

Overall, among all the suspicious microplastic particles found in the gastrointestinal tract, the color distribution was as follows: 36% clear, 35% blue, 15% red, 13% dark or black, and less than 1% green. In the gills, 44% were blue, 37% were clear, and 19% were dark.

Size of the Detected Particles

All suspicious particles in the gills were analyzed and are shown in Figure 6 by size. Of the films, 50% measured between 1 and 2 mm, 25% between 0.1 and 1 mm, 6% between 3 and 4 mm, 6% between 4 and 5 mm, and 13% were larger than 5 mm.

Regarding fibers, 25% were between 1 and 2 mm, 19% smaller than 0.5 mm, 21% between 0.5 and 1 mm, 16% between 2 and 3 mm, 8% between 3 and 4 mm, 7% larger than 5 mm, and 4% between 4 and 5 mm.

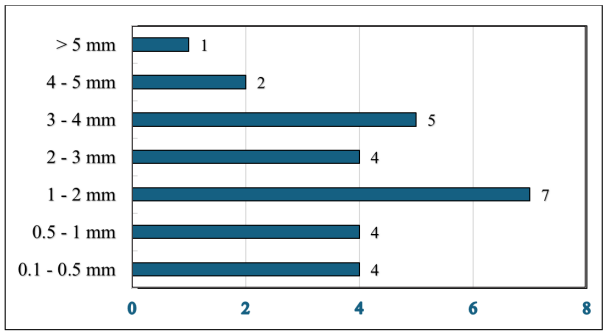

Figure 6) Size of the detected particles in the digestive system and gills.

In the gastrointestinal tract, the sizes of the 142 suspected microplastic particles, categorized by type, are presented below and summarized visually in Figure 7.

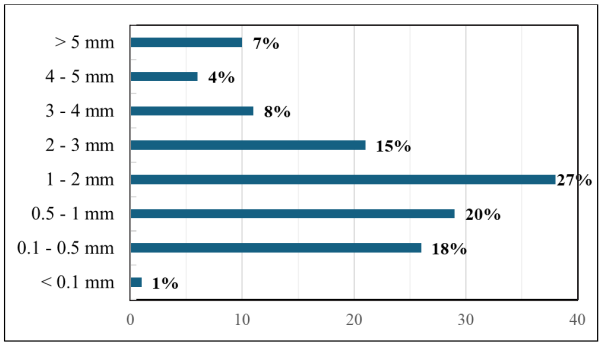

Figure 7) The size of microplastics in the digestive tract.

Identification by FTIR Device

Figure 8 shows a spectrum characteristic of nylon: prominent bands at ~3300 cm^{-1} (N–H stretch, Amide A), ~2920 and ~2850 cm^{-1} (C–H stretch of CH_2), ~1635 cm^{-1} (C=O stretch, Amide I), and ~1535 cm^{-1} (Amide II: N–H bend + C–N stretch). These match standard nylon 6,6 spectra, indicating minimal oxidation.

Figure 9 displays a typical polyethylene (PE) spectrum: strong C–H stretches at 2920 and ~2850 cm^{-1}, CH_2 scissoring at ~1465 cm^{-1}, and CH_2 rocking at ~720 cm^{-1} (single peak, suggesting LDPE or low-crystallinity PE). Absence of carbonyl bands (1700–1750 cm^{-1}) confirms limited weathering.

These polymers (nylon likely from textiles/fishing gear; polyethylene from packaging/films) point to anthropogenic sources in the Oman Sea.

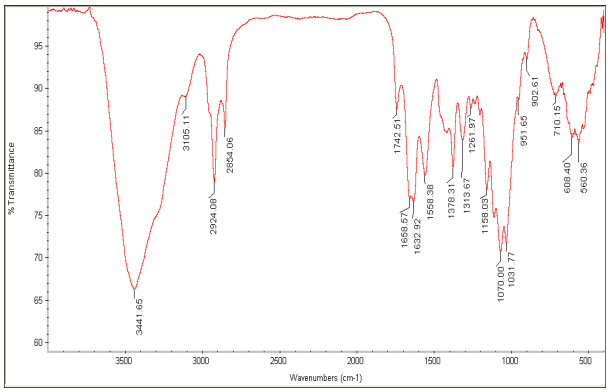

Figure 8) Nylon spectrum identified by the FTIR device from the microplastics sent.

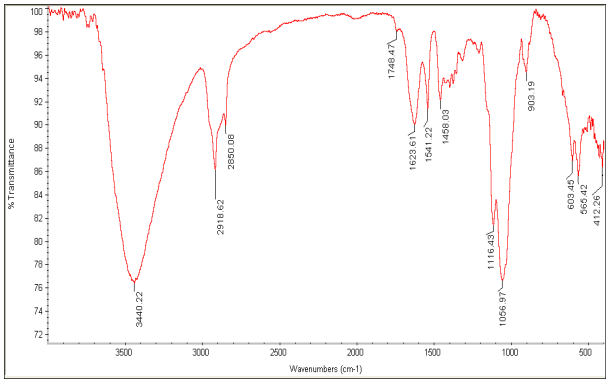

Figure 9) Polyethylene spectrum identified by the FTIR device from the microplastics sent.

Conclusion

Because of the high speed at which tuna move both horizontally and vertically in the water, it is difficult to define a specific habitat for them. Additionally, since the fish's mouth remains open while swimming to supply oxygen, it comes into direct contact with many environmental pollutants. Therefore, tuna can serve as an effective indicator for

studying various pollutants. Moreover, since this fish is canned, distributed worldwide, and easily accessible as a popular food item, it is essential to evaluate its safety from all perspectives. Although it was not possible to investigate the presence or absence of plastic nanoparticles in the fish's blood and muscle tissues due to resource limitations, existing studies suggest they may be present. The results of this study show that microplastics are much more prevalent in the gastrointestinal tract than in the gills. This is likely because water currents introduce pollutants into the environment, which easily pass through the gills. In contrast, the gastrointestinal tract tends to accumulate more due to its limited capacity for excretion. The findings indicate pollution in the waters of the Oman Sea and Chabahar Gulf.

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Authors' Contributions

M Barati Goldareh conducted sampling, laboratory analyses, data processing, and preparation of the initial manuscript draft. **A Shakouri** supervised the study, provided scientific guidance throughout the research, critically revised the manuscript, and approved the final version for publication. Both authors contributed to the interpretation of the findings and approved the final manuscript.

Ethical Permission

Fish specimens used in this study were obtained from commercial fisheries operating in the Oman Sea. No live animals were subjected to experimental procedures, handling, or manipulation for research purposes. Therefore, in accordance with institutional and national guidelines, specific ethical approval was not required for this study.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AI Use Declaration

Artificial intelligence tools were used only minimally to translate some scientific texts between English and Persian. They were not employed in the original writing of the manuscript, study design, data analysis, or interpretation of results. The authors created all scientific content.

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