First Report of Microplastic Ingestion in Edible Fish along Moroccan Mediterranean Coasts

Assia Bouzekry 1, Bilal Mghili 1, Oumayma Bouadil 2, Monique Mancuso 3, Mohamed Ben-Haddad 4, Teresa Bottari 3,* and Mustapha Aksissou 1

Abstract: Monitoring the ingestion of microplastics (MPs) by marine organisms in the environment is essential for understanding the threats posed by these pollutants. In this study, we assessed, for the first time, the presence of MPs in the digestive tracts of two fish species, *Chelon auratus* and *Sardina pilchardus*, as well as a bivalve species, *Callista chione*, in the Moroccan Mediterranean Sea. Moreover, we calculated the polymeric hazard index (PHI) to verify the hazard level of isolated polymers. The results showed that all species ingested MPs, indicating a high level of MP pollution in this area. The MPs ingested were predominantly small (0.5–1 mm) and had a fiber-like shape. The results showed that PP and PET were the most abundant polymers ingested. The highest occurrence of MPs (100%) was found in *C. chione* and *C. auratus*, followed by *S. pilchardus* (72%). In *C. chione*, the average number of ingested MPs was 19.19 items per individual, while in *C. auratus*, the mean abundance of ingested MPs was 19.19 items per individual, while in *C. auratus*, the mean abundance of ingested MPs was 16.82 items per individual, and 9.64 items per individual in *S. pilchardus*. Moreover, the polymer hazard index showed that PET was in hazard level IV, while PP was in level III. Further studies are required in the Moroccan Mediterranean Sea to obtain a better evaluation of the occurrence, distribution, and impacts associated with MP pollution.

Keywords: biomonitoring; ingestion; marine biota; bioindicator; plastic; Alboran Sea

1. Introduction

In recent years, the production and consumption of plastic material has multiplied considerably. This, together with poor waste management, has created a high quantity of plastic waste, which is one of the most common types of anthropogenic pollution in the aquatic environment [1,2]. Plastic debris enters the ocean from a variety of sources, including those originating from activities at sea and on land. Furthermore, it can be transported for long distances before washing up on coastlines or settling on the seafloor [1].

Projections suggest that the production of plastic pollution will reach approximately 710 million tons in 2040 [3]. Alarmingly, by 2050, it is expected that the quantity of plastic debris in the ocean will exceed that of fish [4]. This trend will exacerbate its detrimental effects on ecosystems and marine biodiversity, as well as lead to substantial economic losses [5–8].

Once the plastic debris enters the marine ecosystem, it is progressively degraded physically (e.g., wind, wave actions, and mechanical transformation), chemically (e.g., photo-oxidation by solar radiation), and biologically, generating smaller pieces of plastic [9] called microplastics (MPs) (less than 5 mm in size). The accumulation of MPs in the oceans is
an increasing threat to the aquatic ecosystem’s health [10]. MPs can be accidentally ingested by marine organisms because they mistake them for natural prey or they can be passively consumed during normal feeding behavior [11]. Ingested MPs can produce many negative impacts on marine organisms, including physiological disturbances [12], reproductive dysfunction [12], growth inhibition [13,14], biochemical alterations [15], blocking of the digestive tracts [16], decreased viability, and even mortality [17]. In addition, MPs can be a vector of harmful contaminants such as heavy metals and persistent organic pollutants (POPs) [18]. Finally, MPs can enhance the bioavailability of contaminants to organisms and ecosystems via adsorption and bioaccumulation [18].

Globally, the Mediterranean Sea is one of the regions most severely impacted by plastic pollution [19]. This basin is subjected to high levels of marine litter pollution due to its semi-enclosed nature, surrounded by three densely populated continents with approximately 150 million people, making it a sink for plastic litter [19]. Although it holds only 1% of the world’s water, the Mediterranean is the main hotspot for plastic waste, with 7% of global MPs [20]. Studies conducted in the Mediterranean documented up to 890,000 plastic items per km² [21] most of which are MPs. In the Mediterranean Sea, the ingestion of microplastics was observed in a wide range of marine organisms, including mollusks, zooplankton, fish, seabirds, sea turtles, and cetaceans [22,23]. Moreover, it is important to highlight that there is a shortage of research documenting the MP abundances in marine organisms in the southern Mediterranean compared to the northern regions.

Extending along a coastline of over 500 km, Morocco’s Mediterranean region is widely used for several anthropic uses, such as fishing, shipping, tourism, and recreational activities. The human activities result in various consequences, including the presence of MPs [24]. The degree of pollution by marine litter found along the Moroccan Mediterranean Sea was identified as unacceptable [25–27]. Moroccan waters serve as feeding, refuge, and breeding grounds for numerous marine species (of which several are endangered species) that are important from both a commercial and environmental point of view [28]. Although there was an increase in research concerning MPs in the Moroccan marine environment [25–27,29], our understanding of the presence, ingestion, and consequences of marine litter in marine organisms remains limited. Due to the ubiquitous presence of MPs in the Moroccan Mediterranean and their multitude of effects on marine organisms, continuous monitoring of MPs in water and biota has been recommended [27]. Among the small pelagic fish species, the European sardine (Sardina pilchardus, Walbaum, 1792) is the most commercialized fish in the Moroccan Mediterranean and has accounted for up to 52% of total captures in recent years [30]. These fish represent a significant part of production and biomass and traditionally played a vital role in the ecosystem due to their strong coupling between demersal and pelagic ecosystems, where they are major prey for pelagic predators [31]. Additionally, they are largely distributed throughout the Mediterranean and have a selective feeding strategy [31,32]. The other important commercial fish in the Moroccan Mediterranean is the golden grey mullet, Chelon auratus (Risso, 1810). They are captured all along the Moroccan Mediterranean coast, and they are a considerable part of the local fish market [30]. This fish often enters the estuaries and mouths of rivers. This is a pelagic species that lives in shallow coastal and pelagic areas, at depths between 0 m and 20 m [33]. However, C. auratus usually follows an opportunistic feeding strategy and is marked by regular contact with the sediment, frequently distributed to the whole water column [33]. The smooth clam Callista chione (Linnaeus, 1758) is one of the main clam species commercialized in the Moroccan Mediterranean [34]. This species is largely distributed along the Moroccan Mediterranean coast, where it is caught by artisanal fishery fleets mainly in shallow coastal waters (5–25 m depth) on sandy to muddy bottoms [34]. In the Moroccan market, demand for this bivalve is always high and growing in summer by consumers. This sessile species filters a significant quantity of water and, consequently, absorbs and accumulates a wide range of marine toxins in the marine environment [35]. This means that C. chione is highly exposed to MPs with various densities. The selection of species studied involved organisms from different habitats, i.e., a sessile mollusk (bivalve)
and two pelagic fish (the sardine and golden grey mullet), reflecting environmental conditions in various aquatic compartments. Besides their habitat differences, these species were selected for their feeding behavior (selective or non-selective), which can differently affect the ingestion and presence of MPs in their bodies. In the Mediterranean, the smooth clam and sardine are among the suggested indicators for MPs due to their wide commercial importance, spatial distribution, feeding strategies, and habitat, as well as documented MP ingestion [22].

Given the scarcity of data about MPs ingested by marine species in Morocco, this study aimed (i) to detect, quantify and characterize MPs in three edible species: the two fish species C. auratus and S. pilchardus and the bivalve species C. chione; and (ii) to evaluate the polymer hazard index (PHI) in order to obtain information about the danger of any polymer for the marine environment and human health.

2. Materials and Methods
2.1. Study Area and Samplings

Samplings were conducted during September and October 2022 at three different locations in the Moroccan Mediterranean: Martil (Lat. 35°37′37.266″ N, Long. 5°16′8.886″ W), M’diq (Lat. 35°41′16.341″ N, Long. 5°18′41.121″ W), and Fnideq (Lat. 35°50′23.462″ N, Long. 5°21′1.607″ W) (see Figure 1).

Figure 1. Geographical map of sampling area in the Moroccan Mediterranean.

Fish samples were captured using a trawl measuring 45 m to 60 m in width and 250 m to 300 m in length, with a mesh size of 1 cm. For shellfish specimens, a trawl measuring 1.5 m in length and 1 m in width, with a mesh size of 1.5 cm, was used. Trawling activities at each site were carried out for a duration of 30 min at an approximate speed of 2 knots. The sampling depths ranged from 20 to 60 m for fish and from 10 to 13 m for shellfish. In total, we collected 35 samples of C. auratus, 50 of S. pilchardus, and 46 of C. chione. The fish and shellfish were promptly placed into aluminum bags and transported to the Chemistry Laboratory at the National Institute of Fisheries Research in Tangier, Morocco, using a
cooler maintained at a temperature of 4 °C. Upon arrival at the laboratory, the samples were stored at −30 °C until analysis.

2.2. Microplastic Isolation

The methodology of this study adheres to the most recent recommendations for research on MPs [36]. In the laboratory, prior to the analysis, each sample was gradually thawed overnight at 4 °C. Then, each fish specimen was measured for total length (TL, cm) and total weight (TW, g). The gastrointestinal tract (GIT), from the esophagus to the end of the intestine, was removed, weighed, transferred to a conical glass flask, and treated with a 10% KOH solution at a ratio of 1:5 (w/v). In the same way, each shellfish specimen was measured (shell length, cm) and weighed (whole body). Regarding the shellfish, each individual was carefully dissected, and all flesh, including the digestive system, was weighed and transferred into a glass flask with a 10% KOH (Sigma Aldrich, Merck, Darmstadt, Germany) solution at a ratio of 1:5 (w/v). Each flask was covered with aluminium foil and stirred at 40 °C for 48 h [36]. After this period each sample was placed in a glass cylinder, and a hypersaline 15% NaCl solution was added in order to obtain density separation of the two phases and to collect the floating MPs. Then, the supernatant was transferred to a filtration system with a stainless-steel funnel (YT-330B, Winteam, Hangzhou, China) and filtered through a fiberglass filter (pore size 1 µm, 47 mm, Whatman, Stuttgart Germany). The filter membranes were then placed in Petri dishes and dried at 40 °C for 24 h. After that, the filters were placed in sterile glass Petri dishes and observed under a stereomicroscope. All suspected microparticles were counted, measured, and photographed.

2.3. Quality Control and Preventing Contamination

To avoid contamination during laboratory assays, rigorous precautions were followed according to Bottari et al. [11]. All samples were processed in a room with restricted access, to prevent any accidental external contamination. All workspaces and tools were cleaned with ethanol and filtered deionized water to prevent any particle contamination. Only glassware was used. Operators’ hands and forearms were cleaned before the procedure, and white cotton lab coats (100% cotton), latex gloves, and masks were used during sample handling and processing. All the solutions (Milli-Q water, KOH, NaCl) were pre-filtered at 0.2 µm (Whatman cellulose nitrate membrane filters, diameter of 47 mm) using a vacuum glass filtration apparatus kit prior to use. The procedures were performed under a laminar hood. To mitigate the risk of airborne contamination, procedural blanks were introduced using a 10% KOH solution. In order to maintain accuracy and prevent an overestimation of MP content, filters dampened with moisture were placed in glass Petri dishes and put under the microbiological hood and next to the microscope.

2.4. Polymer Identification

A total of 23 MP particles were randomly selected and analyzed using Raman spectroscopy. The use of Raman spectroscopy for the identification of MPs has recently increased due to the precise characterization and its ability to count micrometer-sized items [11]. Raman spectra were quantified using an HR Evolution micro-confocal Raman system (Horiba Scientific, Kyoto, Japan) with a DXR 532 nm laser diode and a 10× Olympus objective. Spectra were generated with wavenumbers of 600 to 4000 cm⁻¹, while the number of spectra accumulations and the duration of laser exposure ranged from 2 to 20 and from 5 to 20 s, respectively, based on the specific dye in the MP. The laser power was regulated below 5 mW to prevent particle photo-degradation. The spectra obtained were characterized by comparing them with those of standard materials listed in the spectral databases of the Bio-Rad KnowItAll Spectral Library and the Spectral Library of Plastic Particles (SLoPP and SLoPP-E).
2.5. Polymer Hazard Index (PHI)

The polymer hazard index (PHI) was employed to assess the potential threat posed by these MPs to human health. The PHI was calculated with the following formula [37]:

$$\text{PHI} = \sum \text{Pn} \times \text{Sn}$$

where Pn is the percentage of specific polymer types detected in edible fish and Sn is the hazard score of polymers developed by Lithner et al. [37]. The PHI was determined following the method of Nithin et al. [38]. PHI was sorted into different hazard levels as reported by Lithner et al. [37]. Based on the values obtained, five hazard levels were given (I = 0–1; II = 1–10; III = 10–100; IV = 100–1000; and V > 1000).

2.6. Data Analysis

The abundance of MPs in this study was expressed as MPs/individual. Descriptive analysis was performed examining mean MP abundance, standard deviation, and percentage of MP characteristics. The Kruskal–Wallis test was used to compare MP abundance between the three species and between the three sampling stations. Pearson’s Chi-squared was applied to check significant associations between MP size, color, and shape between the three species. Statistical tests were carried out with IBM SPSS Statistics 20 software. Statistical significance was determined at 0.05.

3. Results

3.1. MPs Abundance

A total of 131 specimens were assessed. The length and weight of the golden grey mullet (C. auratus) sampled varied from 20 to 29.2 cm with an average of 22.54 cm and 64.79 to 178 g with a mean of 91.22 g, respectively (Table 1). The European pilchard (S. pilchardus), weight recorded ranged from 54 to 98 g, with an average of 74.35 g, and a length from 19.4 to 24 cm, with an average of 21.52 cm (Table 1). The smooth clam (C. chione), showed a size between 5.5 and 7.8 cm (mean = 6.90 cm) which corresponds to a weight between 10 and 44.23 g (mean = 21.90 g).

The results showed that 1.954 suspected items were ingested/accumulated by the three species: 883 in C. chione, 589 in C. auratus, and 482 in S. pilchardus. The photographs of MPs detected in fish and bivalves are shown in Figure 2.

Regarding fish, C. auratus showed 100% (n = 35) MP ingestion, while S. pilchardus showed 72% (n = 32, Table 1).

In C. chione, the average number of ingested/accumulated MPs was 19.19 items/individual. For the fish species, C. auratus exhibited an average of 16.82 items/individual, while S. pilchardus had an average of 9.64 items/individual.

The Kruskal–Wallis test indicated that there were no significant differences in the number of ingested MPs among the three species (H = 2, p > 0.05).
The highest frequency of occurrence was observed in the bivalve *C. chione* (100%, \( n = 46 \)). Regarding fish, *C. auratus* showed 100% (\( n = 35 \)) MP ingestion, while *S. pilchardus* showed 72% (\( n = 32 \), Table 1).

In *C. chione*, the average number of ingested/accumulated MPs was 19.19 items/individual. For the fish species, *C. auratus* exhibited an average of 16.82 items/individual, while *S. pilchardus* had an average of 9.64 items/individual.

The Kruskal–Wallis test indicated that there were no significant differences in the number of ingested MPs among the three species (\( H = 2, p > 0.05 \)).

*C. chione* specimens collected from the Martil station showed the highest number of MPs per individual (22.52 items/individual). Finally, specimens collected from the M’diq and Fnideq stations showed a significant MP abundance of 18.37 items/individual and 16.28 items/individual, respectively.

In the case of *S. pilchardus*, the abundance of ingested MPs was higher in samples from the Fnideq station (15.85 items/individual) than in the M’diq station (9.06 items/individual).

The percentage of ingested/accumulated MPs, size, shape, and color categories did not differ between the three species (Pearson’s chi-square test, \( p > 0.05 \)).

The microplastics detected in the examined marine organisms exhibited a range of shapes, with fibers being the most common at 80.60%, followed by fragments (17.70%) and foam (1.70%). No pellets or films were found. Fibers were dominant for both fish and bivalve species (93–95% and 65%, respectively) (Figure 3). Fragments constituted 31% in bivalve samples and between 5 and 7% in fish samples. Foams were only observed in *C. chione* specimens sampled in the M’diq and Fnideq stations (1.31 and 10.20%, respectively).

Regarding the colors, more than half of MPs were found to be black (56.34%), followed by yellow (12.43%), blue (11.05%), red (8.80%), transparent (4%), grey (2.91%), violet (1.07%), green (0.61%), and orange (0.10%) (Figure 4). The black color was predominant in all three species: 72.36% in *C. auratus*, 60.80% in *S. pilchardus*, and 43% for *C. chione*.

The MPs detected in the three species were categorized into five groups: 0.5–1 mm, 1–2 mm, 2–3 mm, 3–4 mm, and 4–5 mm. The overall average size of MPs recorded in this study was 0.91 mm. Among the size classes observed, MPs within the 0.5–1 mm range were the most prevalent, constituting 68.83% of the total. This was followed by 1–2 mm (20.90%), 2–3 mm (6.34%), 3–4 mm (2.76%), and 4–5 mm (1.17%) (Figure 5a). This study highlights that MPs ranging from 0.5 to 1 mm were very abundant at all the sampling sites, Martil (77.46%), M’diq (64.13%), and Fnideq (60.36%). However, 4–5 mm sized MPs were high in the M’diq station, with 3.18% (Figure 5).
Remarkably, bivalve specimens tended to ingest/accumulate larger sizes of MPs than the average size in the two fish species. Results showed that the most represented MP size category was the 0.5–1 mm range, followed by 1–2 mm, 2–3 mm, 3–4 mm, and 4–5 mm. The overall average size of MPs recorded in this study was 0.91 mm. Among the size classes observed, MPs within the 0.5–1 mm range were the most prevalent, constituting 68.83% of the total. This was followed by 1–2 mm, 2–3 mm, 3–4 mm, and 4–5 mm (20.90%, 6.34%, 2.76%, and 1.17%, respectively) (Figure 5).

Regarding the colors, more than half of MPs were found to be black (56.34%), followed by yellow (12.43%), blue (11.05%), red (8.80%), transparent (4%), grey (2.91%), and violet (0.10%) (Figure 4). The black color was predominant in all three species: 72.36% in C. auratus, 60.80% in S. pilchardus, and 43% in C. chione (Figure 5a). No significant differences in the size of the MP were high in the M’diq station, with 3.18% (Figure 5).

MP polymers detected in edible fish species were Polypropylene (PP), Polyethylene terephthalate (PET), Polyvinyl chloride (PVC), Polyethylene (PE), and polycarbonate (PC). Examples of MPs observed in the biota studied with the corresponding Raman spectra are shown in Figure A1. As the assay performed by Raman spectroscopy identified these types of plastic polyesters.

Figure 3. Percentage of MP shapes representing all three species (a) and among species (b).

Figure 4. Percentage of MP colors representing all three species (a) and among species (b).

Figure 5. Percentage of MP sizes representing all three species (a) and among species (b).
Results showed that the most represented MP size category was the 0.5–1 mm range, with a percentage of 71.18% in \textit{C. auratus}, 69.08% in \textit{S. pilchardus}, and 63.95% in \textit{C. chione}. Remarkably, bivalve specimens tended to ingest/accumulate larger sizes of MPs than the fish (3–5 mm). The average size of MPs observed in \textit{C. chione} was 0.95 mm, while the average size in the two fish species was 0.86 mm. No significant differences in the size of the MPs ingested were observed between the three species according to the Kruskal–Wallis test ($p < 0.05$).

3.2. Polymer Composition and Risk Assessment

The assay performed by Raman spectroscopy identified five types of plastic polymers. These include Polypropylene (PP), Polyethylene terephthalate (PET), Polyvinyl chloride (PVC), Polyethylene (PE), and polycarbonate (PC). Examples of MPs observed in the biota studied with the corresponding Raman spectra are shown in Figure A1. As shown in Figure 6, the most prevalent polymer detected was PP (39.14%), followed by PET (26.08%), PVC (17.40%), PE (8.69%), and PC (8.69%).

PHI values for the identified polymers ranged from 10.22 (PC) to 183.46 (PVC), with a total PHI of 432.72, indicating a hazard level of IV (100–1000). Only PP (39.13) was in the moderate risk category (Table 2). PE (95.59) and PP had a PHI ranging from 10 to 100, placing them in the hazard category III, which denotes a high risk. Finally, PVC (183.46) and PET (104.32) had a PHI ranging from 100 to 1000, placing them in the hazard category IV.

Table 2. Polymers detected are categorized according to hazard level and polymer hazard index (PHI).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Monomer</th>
<th>Hazard</th>
<th>Hazard Level</th>
<th>Score</th>
<th>PHI</th>
<th>Hazard Category</th>
<th>Risk Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>Propylene</td>
<td>Harmful when inhaled.</td>
<td>I</td>
<td>1</td>
<td>39.13</td>
<td>III</td>
<td>High</td>
</tr>
<tr>
<td>PET</td>
<td>Ethylene glycol</td>
<td>Harmful if swallowed.</td>
<td>II</td>
<td>4</td>
<td>104.32</td>
<td>IV</td>
<td>Very High</td>
</tr>
<tr>
<td>PVC</td>
<td>Vinyl chloride</td>
<td>Extremely flammable gas; May cause cancer.</td>
<td>V</td>
<td>10.5</td>
<td>183.46</td>
<td>IV</td>
<td>Very High</td>
</tr>
</tbody>
</table>
Table 2. Cont.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Monomer</th>
<th>Hazard Description</th>
<th>Hazard Level</th>
<th>Score</th>
<th>PHI</th>
<th>Hazard Category</th>
<th>Risk Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>Ethylene</td>
<td>Extremely flammable gas; May cause drowsiness or dizziness.</td>
<td>II</td>
<td>11</td>
<td>95.59</td>
<td>III</td>
<td>High</td>
</tr>
<tr>
<td>PC</td>
<td>Bisphenol A</td>
<td>May cause an allergic skin reaction; Suspected of damaging fertility or the unborn child; May cause drowsiness or dizziness.</td>
<td>IV</td>
<td>1.2</td>
<td>10.22</td>
<td>II</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

4. Discussion

The rate of plastic pollution along the Moroccan Mediterranean coast is rising alarmingly [28], but, currently, knowledge about the ingestion of microplastics by marine organisms is limited. This study aimed to investigate the occurrence, abundance, and chemical composition of MPs ingested/accumulated by three edible marine organisms at three locations in the Moroccan Mediterranean Sea. The presence of hazardous polymers for human and marine environment was also studied by calculating the PHI.

Although the number of microplastics examined and characterized is limited, this is the first study conducted on fish and shellfish along the Moroccan Mediterranean coastline. These initial findings are very important as not only do they shed light on the presence of this pollution, but they also offer valuable information on the type of pollutants present.

The abundance of MPs varied among the three species. The habitat preferences of marine biota could influence the ingestion of MPs [39]. Based on their habitat preferences, *C. auratus* and *S. pilchardus* were categorized as pelagic fish, while *C. chione* was classified as a benthic species. The highest concentrations of MPs were observed in *C. chione* (19.19 MPs/ind). This result is expected since bivalves feed through sediment filtration, resulting in a higher intake of MP particles, linked to the fact that MPs/NPs tend to sink to the seafloor [40,41]. Bivalve species possess the ability to filter and accumulate MPs of various sizes, with the levels depending on the abundance and distribution of MPs in the water [40]. *C. chione* is a shallow-burrowing benthic suspension feeder [34,35] that lives on sandy bottoms in coastal waters at depths varying from 1 to 180 m, although it is more common between 5 and 10 m depths on the Martil coast and other regions studied [34]. Several studies highlighted the presence of high concentrations of MPs in bivalves, especially in edible clams, mussels, and oysters [40–43]. The average number of ingested MPs in *C. chione* (19.19 items/individual) is similar to the values recorded in bivalves in China (24.64 items/individual, Wu et al. [44]), Iran (21.8 items/individual, Abbasi et al. [45]), and Vietnam (30.67 items/individual, Thanh et al. [46]). However, the values found were higher than those observed by Rios-Fuster et al. [47] in the western Mediterranean (4.83 items/individual), in Spain (4.54–18.2 items/individual, Capo et al. [40]), Tunisia (7.7 items/individual, Wakkaf et al. [48]), and Italy (3.95 ± 2.54 items/individual, Nalbone et al. [41]). Finally, our results are lower than values recorded in India (91.42 items/individual, Naidu et al. [43]) and in the Mediterranean coasts of Turkey (39.15 items/individual, Atici [42]. Due to their feed habits and habitat, this group of invertebrates offers optimal ecological characteristics to be a bioindicator species for monitoring MPs in the marine environment [48].

The intake and ingestion of MPs can differ among marine species due to the variations in their feeding behavior. *C. chione* is a filter-feeder and can accidentally ingest suspended items, so this non-selective feeding behavior could explain the high levels of MPs found in this study. In our study, *C. auratus* was found to have the highest quantity of MPs in their digestive tract compared to *S. pilchardus*. The diet of *C. auratus* consists of small benthic organisms, unicellular algae, and occasionally plankton [33]. This opportunistic feeding strategy used by *C. auratus* may expose them to high levels of MP ingestion. This
aspect makes *C. auratus* more prone to ingesting MPs dispersed throughout the water column. Furthermore, *C. auratus* enters and feeds near rivers, increasing the risk of MP ingestion. Depending on their feeding strategies, European sardines have a highly selective diet [31,32] and rarely consume MPs, which explains the low percentage of ingestion by this species compared to *C. auratus*. It is the only species, among the examined animals, that feeds exclusively on pelagic organisms. The differences in ingestion and intake of MPs between the three species may have been caused by variations in the concentration of MPs in the surrounding water in which the species live. However, by decreasing the MP size, fish ingestion can rise due to their incapacity to differentiate between MPs and food [44]. The method of contamination probably plays an important role in the differences observed in the three species. For example, the fish’s original prey may already be polluted with MP via false ingestion, secondary consumption, or trophic transfer [49]. However, it should not be forgotten that MPs ingested by these marine species can accumulate in predatory species that eat these contaminated species [50]. Bioaccumulation of MPs happens when their uptake from the marine ecosystems by all possible routes—i.e., ingestion and contact, from all possible sources—i.e., sediments, water, or prey [50]. Bioaccumulation and subsequent trophic transfer of MPs can lead to biomagnification of these pollutants at higher trophic levels [50]. MPs can also increase the bioavailability of contaminants to marine organisms through bioaccumulation [18].

The occurrence of MPs in *C. auratus* was studied by a limited number of researchers (Table 3). Güven et al. [51] showed an average of 7.47 items/individual, similar to values (6.66 items/individual) found in Egypt (Mediterranean Sea) [52] (Table 3), while in Tunisia, Abidli et al. [53] recorded the presence of MPs in 87% of *C. auratus* specimens studied, with values higher than our results (65.33 items/individual in the Bizerte lagoon and 42 items/individual in the Ghar El Melh lagoon). In this study, MP abundances in *S. pilchardus* (9.64 items/individual) were higher than those recorded by Filgueiras et al. [54] (1.77 items/individual) in the NW Iberian shelf, and by Rios-Fuster et al. [47] (0.44 items/individual) in Spain. Also, Kermenidou et al. [55] reported lower values than our results (2.5 items/individual) in the Thermaic Gulf (North Aegean Sea), as well as Savoca et al. [56], who detected 0.53 items/individual in the Southern Tyrrenian Sea, and Anastasopoulou et al. [57], who reported 0.8 items/individual in the Eastern Ionian Sea (see Table 3).

By contrast, the MP concentrations recorded in *S. pilchardus* were comparable to those recorded in the same species in the Adriatic Sea (4.63 items/individual), Italy (6.21 items/individual, Trani et al. [58]), and Spain (8.62 items/individual, Sánchez-Guerrero-Hernández et al. [59]).

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### Table 3. Summary of microplastic concentrations in *Chelon auratus* and *Sardina pilchardus* along the Mediterranean coasts.

<table>
<thead>
<tr>
<th>Sampling Area</th>
<th>Species</th>
<th>N</th>
<th>FO (%)</th>
<th>N Items/Individual</th>
<th>Polymer</th>
<th>Identification Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern Ionian Sea</td>
<td><em>Chelon auratus</em></td>
<td>20</td>
<td>95</td>
<td>9.5</td>
<td>-</td>
<td>-</td>
<td>Anastasopoulou et al. [57]</td>
</tr>
<tr>
<td>Northern Levant Sea</td>
<td><em>Chelon auratus</em></td>
<td>39</td>
<td>36</td>
<td>3</td>
<td>PE, PP</td>
<td>FT-IR</td>
<td>Güven et al. [51]</td>
</tr>
<tr>
<td>Northern Tunisia</td>
<td><em>Chelon auratus</em></td>
<td>10</td>
<td>-</td>
<td>65.3</td>
<td>PP, PE</td>
<td>FT-IR</td>
<td>Abidli et al. [53]</td>
</tr>
<tr>
<td>Northern Adriatic Sea</td>
<td><em>Sardina pilchardus</em></td>
<td>10</td>
<td>25</td>
<td>1.4</td>
<td>PVC, PS, PP</td>
<td>FT-IR</td>
<td>Avio et al. [60]</td>
</tr>
<tr>
<td>Alboran Sea; Northern Spain; Gulf of Lion</td>
<td><em>Sardina pilchardus</em></td>
<td>105</td>
<td>33.3</td>
<td>0–0.5</td>
<td>PET, PA, PE, cellophane, cotton, wool</td>
<td>FTIR</td>
<td>Compa et al. [61]</td>
</tr>
</tbody>
</table>

---
### Table 3. Cont.

<table>
<thead>
<tr>
<th>Sampling Area</th>
<th>Species</th>
<th>N</th>
<th>FO (%)</th>
<th>N Items/Individual</th>
<th>Polymer</th>
<th>Identification Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern Adriatic Sea</td>
<td><em>Sardina pilchardus</em></td>
<td>99</td>
<td>19</td>
<td>1.8</td>
<td>PE, PET, PS, PVC, Nylon</td>
<td>FT-IR</td>
<td>Avio et al. [62]</td>
</tr>
<tr>
<td>Northern Adriatic Sea</td>
<td><em>Sardina pilchardus</em></td>
<td>80</td>
<td>96</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Renzi et al. [63]</td>
</tr>
<tr>
<td>Northern Adriatic Sea</td>
<td><em>Sardina pilchardus</em></td>
<td>30</td>
<td>37</td>
<td>0.9</td>
<td>-</td>
<td>-</td>
<td>Anastasopoulou et al. [57]</td>
</tr>
<tr>
<td>Eastern Ionian Sea</td>
<td><em>Sardina pilchardus</em></td>
<td>36</td>
<td>47</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>Anastasopoulou et al. [57]</td>
</tr>
<tr>
<td>Northern Levant Sea</td>
<td><em>Sardina pilchardus</em></td>
<td>7</td>
<td>-</td>
<td>2.7</td>
<td>PE, PP</td>
<td>FT-IR</td>
<td>Güven et al. [51]</td>
</tr>
<tr>
<td>Northern Spain</td>
<td><em>Sardina pilchardus</em></td>
<td>15</td>
<td>87</td>
<td>1.8</td>
<td>PE, PP</td>
<td>μRaman</td>
<td>Filgueiras et al. [54]</td>
</tr>
<tr>
<td>Northern Spain</td>
<td><em>Sardina pilchardus</em></td>
<td>7</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
<td>Rios-Fuster et al. [47]</td>
</tr>
<tr>
<td>Northern Spain; Gulf of Lion</td>
<td><em>Sardina pilchardus</em></td>
<td>104</td>
<td>58</td>
<td>1.1–1.8</td>
<td>-</td>
<td>-</td>
<td>Pennino et al. [64]</td>
</tr>
<tr>
<td>Gulf of Lion</td>
<td><em>Sardina pilchardus</em></td>
<td>13</td>
<td>-</td>
<td>0.5</td>
<td>PE</td>
<td>FT-IR</td>
<td>Constant et al. [23]</td>
</tr>
<tr>
<td>North Aegean Sea</td>
<td><em>Sardina pilchardus</em></td>
<td>-</td>
<td>-</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
<td>Kermenidou et al. [55]</td>
</tr>
<tr>
<td>Southern Tyrrhenian Sea</td>
<td><em>Sardina pilchardus</em></td>
<td>19</td>
<td>-</td>
<td>0.5</td>
<td>PP, PAN, PE, PA</td>
<td>Raman, FT-IR</td>
<td>Savoca et al. [56]</td>
</tr>
</tbody>
</table>

Frequency of occurrence: FO; Number of specimens: N; Polyethylene: PE; Polypropylene: PP; Polyvinyl Chloride: PVC; Polyamide: PA; Polyacrylonitrile: PAN; Polystyrene: PS; Polyethylene terephthalate: PET.

Differences between MP abundances recorded in the Moroccan Mediterranean Sea compared to other studies may be due to several factors. Firstly, these differences may arise from variations in the methods used for MP extraction. Secondly, the varying levels of MP pollution in the study sites and the subsequent ingestion of microplastics by marine organisms could be contributing factors. For instance, certain areas might exhibit a higher concentration of wastewater treatment plants [65], estuaries [66], or the influence of large coastal cities [24,65], all of which have a notable impact on the marine environment.

The three locations selected for our study are severely affected by tourism, as previously reported by Mghili et al. [29], who classified them as extremely dirty. In fact, off the coasts of the Moroccan Mediterranean Sea, there are several wastewater treatment plants discharging into the sea. MP ingestion and intake incidence was higher in samples from Martil than in Fnideq and M’diq, indicating a relationship between MP bioavailability and proximity to urban centers. In addition, Martil receives a high amount of input from the Martil River, and this could explain the high number of MPs found in the studied species in this region. Moreover, the high abundance of MPs in the Moroccan beach sediments provides further evidence of the level of pollution in this region. In addition, the high abundance of plastic litter on the seafloor, regularly observed in bottom trawl fishing, could explain the rising incidences of ingestion of MPs in the three examined species [27]. Also, Alshawafi et al. [24] indicated that the highest concentrations of MPs were recorded near the most populated metropolitan areas in the Moroccan Mediterranean. A strong correlation between MP density in water and MP density in marine organisms has already been reported [66].
The ingestion of MPs by marine species is affected by their sizes, shapes, and colors, as well as by their source [67]. Our findings indicated that the major MP shape found was fiber. This result is in accordance with the studies conducted in the Iberian Peninsula by Bellas et al. [68], Compa et al. [61], Lopes et al. [69], Filgueiras et al. [54], and Pennino et al. [64], as well as in Portugal by Neves et al. [70], where fibers constitute over 60% of the MPs found. A similar pattern was observed in studies conducted in Tunisia [53] and Egypt [52]. Several studies revealed that fibers are the most common in the ecosystem marine, such as sea surface waters, seabeds, and in the gastrointestinal tracts of invertebrates and vertebrates [22,57,68]. Microfibers were reported to be widely ingested by aquatic species because they are often mistaken as prey [71]. In our study, we also found high levels of fiber ingestion. These fibers are probably a consequence of the degradation of ropes and fishing nets, as well as the washing of synthetic clothing [72]. These synthetic textiles account for 35% of the global release of primary microplastics into the marine environment [72], and can be ingested by aquatic biota [8], as also reported in recent studies that highlighted the MPs' ubiquity in the Moroccan marine environment [24].

Regarding the color of microplastics, it plays a crucial role in influencing the probability of ingestion by marine organisms. In our study, we observed that black and blue were the dominant color in all three species, constituting a range of 12–63%. Our results are supported by previous studies, such as those reported by Abidli et al. [53] in Tunisia, Hamed et al. [73] in Egypt, and Kılıç et al. [74] in Turkey, as well as, Bellas et al. [68], on the Spanish Mediterranean and Atlantic coasts. Moreover, our findings are in accordance with Matluba et al. [75] and Zhang et al. [59] in Bangladesh and in the East China Sea, respectively. Compa et al. [61], on the Mediterranean coast of the Iberian Peninsula, showed that blue and transparent particles were the most frequent colors. On the contrary, Filgueiras et al. [54] highlighted that sardine and anchovy specimens from the western Cantabrian Sea ingested a predominance of transparent items. These differences may be attributed to the prevalence of these colors in the environment or to the selectivity in targeting particles that resemble their natural prey [67].

Moreover, this study highlighted that small MPs were the most dominant in all three species (0.5–1 mm), as previously reported by Bellas et al. [68] in fish species from the Spanish Mediterranean and Atlantic coasts, and Filgueiras et al. [54] in pelagic and benthic fish from the northwestern Iberian Plateau. The average size of MPs obtained in the Gulf of Cadiz by Sánchez-Guerrero-Hernández et al. [59] was 0.92 mm for sardines and 0.88 mm for anchovies. Similarly, Lopes et al. [69] observed the dominance of the size class below 0.50 mm, while Filgueiras et al. [54] reported slightly higher average sizes (i.e., 1.46 mm for sardines), and the most common size class was 0.5–1 mm. Finally, the average size of MPs in fish and water in the South China Sea was less than 0.5 mm [71].

The polymer characterization performed by Raman showed a total of five polymer types: PP, PET, PVC, PE, and PC. These polymers have been recognized as the most common plastics contaminating the marine environment due to their wide range of applications and durability [76]. This finding is in line with what Suaria et al. [20] reported, highlighting the prevalence of PP (polypropylene) and PE (polyethylene) in the Mediterranean surface waters [77] and emphasizing that these two polymers represent 62% of global plastic demand. Similar findings were also recorded by Abidli et al. [53] in fish in Tunisia and by Neves et al. [70] in commercial fish off the Portuguese coast. One of the most likely sources of MPs (PP and PET) in water environments is fishing gear. The use of plastic ropes and fishing nets in the Moroccan Mediterranean coasts is a probable source of MPs in the marine biota in this region. Discarded clothing also contributes to MP pollution in Moroccan waters. PP and PE microfibers have been linked to textile washing activities.

Finally, the PHI results showed that PVC and PET had the highest PHI, indicating that their release into the water could have serious harmful effects not only on marine life but also on human health [38]. In contrast, PP had the lowest PHI, suggesting that it represents a relatively lower risk. Although the hazard of ingesting MPs is high, our results are preliminary, and further studies on PHI are needed to better understand the
potential risk to human and marine environmental health. In Morocco, fish represent the most important source of protein consumed by the population and, therefore, can serve as the main exposure route of MPs and associated pollutants for many people. Sardines remain the most important fishery product, with national production surpassing 850,000 tonnes per year, being the most common species in Morocco in terms of biomass, catches, and consumption. Finally, sardines represent more than half of total Moroccan fisheries’ production (52%) [30]. Similarly, *C. chione* is a commercially important species on many coasts of the Moroccan Mediterranean. Due to the significant consumption of this species, the presence of microplastics in bivalves poses a potential risk to human health. In particular, most species of bivalves are consumed whole, which can increase human exposure to MPs. In contrast, the risk of ingestion and intake of MPs in fish is mitigated by the removal of the gastrointestinal tract in most species. However, when humans consume polluted species, it can result in the bioaccumulation of MP [50]. Moreover, the exposure to chemical contaminants associated with MPs has become a serious issue, given that chemical bioaccumulation in marine biota can reach human consumers [18].

One of the main limitations of this study is the low percentage of MPs analyzed by Raman spectrometry. Further studies would be required for more vigorous detection of MP concentrations. To achieve these objectives, it is important to use more specific technology (i.e., µ-FTIR and µ-Raman) in future studies in Morocco to detect small-sized MPs.

5. Conclusions

This study assesses, for the first time, the contamination by MPs in three edible marine organisms off the coast of Tetouan in the Mediterranean Sea. The observed concentrations significantly exceed those reported in prior studies conducted in the Mediterranean Sea. The bivalve *C. chione* was more sensitive to MP intake than others, suggesting that the different behavior strategies and feeding play a crucial role in determining the ingestion of these contaminants. The main limitations of this study include its restricted sampling period, which does not consider seasonal variations, and the lack of comparative studies, which prevents the investigation of correlations between MP abundance in biota and in water and sediments. This paper also highlights the occurrence and potential risks of MPs in ocean food webs and for human health. It emphasizes the need for additional studies on the sources of MP pollution and the impact of these pollutants on marine species. In light of these findings, it is strongly recommended to expand the scope of research to encompass a broader array of species and habitats, facilitating a comprehensive assessment of MP pollution concentrations in marine organisms within the Moroccan Mediterranean. Additionally, measures should be implemented to prevent the accumulation of MP pollutants in Moroccan coastal water.

**Author Contributions:** Conceptualization, A.B. and B.M.; Formal analysis, A.B., B.M. and M.B.-H.; Methodology, A.B., B.M., O.B. and T.B.; Supervision, M.M., T.B. and M.A.; Validation, B.M., M.B.-H. and M.A.; Writing—original draft, A.B., B.M., O.B. and M.M.; Writing—review and editing, B.M., M.M. and T.B. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.
Appendix A

![Graph A](image1.png)

![Graph B](image2.png)

**Figure A1.** Examples of Raman spectra of samples analyzed. (a): Polypropylene and (b): Polyvinyl Chloride.

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