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# Microplastic occurrence in the gastrointestinal tract and gill of bioindicator fish species in the northeastern Mediterranean

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ABSTRACT

Microplastic pollution is an extremely emerging problem and its potential threats to the aquatic organisms were investigated worldwide. In this study, four different commercial fish species (*Mullus barbatus* (Linnaeus, 1758), *Mullus surmuletus* (Linnaeus, 1758), *Mugil cephalus* (Linnaeus, 1758), *Saurida undosquamis* (Richardson, 1848)) were used as a bioindicator to assess the microplastic pollution in the northeastern Mediterranean. The frequency of occurrence in the gastrointestinal tract (GIT) and gill was varied between 66–100% and 68–90%, respectively. The highest microplastic abundance was detected at the GIT of *M. cephalus* sampled from Asi River estuary. The majority of extracted microplastics were fiber, black in color and less than 1 mm in size. Fourier Transform Infrared Spectroscopy (FTIR) indicated the most common polymer type as polyethylene. This study is the first study examining the microplastic existence in gill and results obtained in this study improve the knowledge about the relationship of microplastic ingestion in fish and environmental conditions in the Northeastern Mediterranean Sea.

## 1. Introduction

Microplastics (MP) are defined as plastic particles which have a smaller size than 5 mm (Arthur et al., 2009). These particles are sometimes produced in microscopic size range or sometimes fragment from larger plastic particles (Habib and Tieaman, 2021). Today, the existence of microplastic particles was reported from the deepest part of the ocean, Mariana Trench, (Peng et al., 2018) to the highest part of the earth, Everest Mountain (Napper et al., 2020).

Microplastic particles reach to the marine environments by river discharges (Constant et al., 2020; Pojar et al., 2021), wastewater treatment plant effluents (Gündoğdu et al., 2018; Habib et al., 2020), and atmospheric deposition (Ding et al., 2021). Marine organisms may feed on them due to their small size and floating properties (Anderson et al., 2016). So far, MP ingestion was reported in zooplankton (Beer et al., 2018; Sun et al., 2018), bivalve (Wu et al., 2020; Yozukmaz, 2021), crustesea (Abbasi et al., 2018; Wu et al., 2020) and fish (Gündoğdu et al., 2020; Aytan et al., 2021). MP ingestion may harm marine organisms in many ways. Firstly, ingested MPs may result in congestion in the digestive system (Walkinshaw et al., 2020). Secondly, nano and/or micro size particles can be absorbed in the intestine and other tissues (Abbasi et al., 2018) which lead to accumulation in different organs (Ivleva et al., 2017). Finally, they also provide sorption sites for chemical pollutants which may lead to the entrance of chemical pollutants into the body of marine organisms (Tien et al., 2020). Since marine organisms and humans are connected with each other via the food chain, MP pollution in the marine environment also poses a risk to human health.

The Marine Strategy Framework Directive was encouraged EU member states to obtain a good ecological status in terms of plastic and microplastic density in marine environments (European Commission, 2010). Since then, not only the determination of microplastic density in the marine environments but also the potential impacts of ingested microplastic particles on the marine organisms have been an up-to-date concern.

The Mediterranean Sea is a semi-enclosed water body with variety of fresh water input (GRID-Arendal, 2013). High urbanized coastal regions surrounded by many pollution sources such as wastewater treatment facilities, industrial discharges, agricultural runoff, intense shipping, and fishing activities cause significant pollution problems (Suaria et al., 2016). More than 80% of marine debris in the Mediterranean Sea was consists of plastics (Olguner et al., 2018; Saladié and Bustamante, 2021; Yılmaz et al., 2022). As a result, it was considered like a microplastic soup due to its high microplastic content (Suaria et al., 2016).

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Bioindicator organisms such as anemone (Morais et al., 2020), shrimp (Nan et al., 2020), crap (Patria et al., 2020), fish (Pegado et al., 2021; Macieira et al., 2021) have been successfully applied to monitor microplastic pollution status of marine environments. Suitability of a fish as a bioindicator was determined depend on fish's occurrence, vagility, commercial value, habitat, distribution (C. Zhang et al., 2020). Previous studies proposed the usage of *Mullus barbatus*, *Mullus surmuletus* and *Mugil cephalus* as an indicator after the consideration of mentioned standards (Güven et al., 2017; C. Zhang et al., 2020). For these reasons, these species were employed as bioindicator in this study. Also, to test the prey predator relationship, *Saurida undosquamis* which was fed with *Mullus surmuletus* was used as bioindicator.

There are some studies investigating the microplastic existence in the gastrointestinal tracts (GIT) of fish in the northeastern Mediterranean coast of Turkey (Güven et al., 2017; Gündoğdu et al., 2020) but, studies conducted in the river estuaries were limited. Asi River discharges its water into Samandağ shelf waters and creates waste deposition peeks at the seabed (Yılmaz et al., 2020). Similarly, higher MP amount in the marine debris was reported in the river mouths (Gündoğdu and Çevik, 2017). The first objective of this study was to test the existence in the MPs presence in the GIT and gills of marine animals obtained from Iskenderun Bay and Samandağ coastal waters. For that purpose, *Mullus barbatus*, *Mullus surmuletus*, *Saurida undosquamis* were selected as indicator species. Second objective was to evaluate of the impact of plastic deposition peeks located at Asi River estuary. For that manner, MP presence in the GIT and gill of *Mugil cephalus* was investigated. This study is, as far as we are aware, the first study conducting at the Samandağ coast and is the first study examining the MP existence in the gill at the northeastern Mediterranean coast.

## 2. Material and methods

### *2.1. Study area*

Iskenderun Bay is highly urbanized region which hosts many economically important ports in Turkey. Therefore, it suffers from a variety of pollutants resulting from port operations, agricultural activities, industrial facilities and fishing activities (Fig. 1). Recent studies indicated a considerable amount of increase in the plastic debris in Iskenderun Bay (Gündoğdu and Çevik, 2017; Büyükdeveci and Gündoğdu, 2021; Yılmaz et al., 2022). On the other hand, Samandağ receives a significant pollution load from the Asi River deriving from many diffuse and point pollution sources (Kilic et al., 2018). It is estimated that plastic waste materials discharged from the Asi River are transported via local currents and create waste deposition peeks on the seabed (Yılmaz et al., 2020). In addition, more than half of the plastic materials collected from Samandağ was of foreign origin and transferred with local currents and effective wind systems (Yılmaz et al., 2022). A recent study indicated that amount of plastic debris on the seabed of Asi River estuary was higher than that of Iskenderun Bay (Yılmaz et al., 2022).

### *2.2. Sampling*

Fish samples were obtained from local fishermen on November 2022. All of the examined fish were recently caught and free of any morphological deformation. Samples were wrapped with tin foil, placed in ice bag and transported to the laboratory. Then, samples were frozen at the −20 °C until further analysis.

### *2.3. Microplastic extraction*

Before any analysis, weight (to the nearest 0.1 g) and total length (to the nearest 0.5 cm) of each specimen were recorded (Table 1). Then, fish were cleaned with pure water. Gastrointestinal tracts (GIT) from the upper part of the oesophagus to the anal opening (Lusher et al., 2017) and gill of each specimen were dissected, weighted (nearest 0.1 g) and placed into a glass beaker, separately. After then, the glass beakers were covered with tin foil. Next, for GIT samples, 20 mL of 30% $H_2O_2$ per gram of organ were added into a glass beaker (Renzi et al., 2019), covered with tin foil and heated on a hot plate until the organic material was degraded (Anastasopoulou et al., 2018). Lastly, final solution was filtered with the use of 50 μm pore size filters. After filtration, filter papers were placed into sterile petri dishes, covered and set aside until microscopic examination.

GIT samples extracted from the *Mugil cephalus* was contained intense sand particles. For that reason, modified methodology was used for sand containing GIT samples. Same methodology was also applied to gill samples. For those samples, salt treatment was applied before filtration. 400 mL saturated sodium chloride solution (1.2 g/mL NaCl) were added into dissolved solutions and mixed with a glass rod (Jabeen et al., 2017). Solutions were transferred into separation funnels and left for 1 day. After density separation, the remaining supernatant was filtered with the use of 50 μm pore size filters. Then, filters were placed into sterile

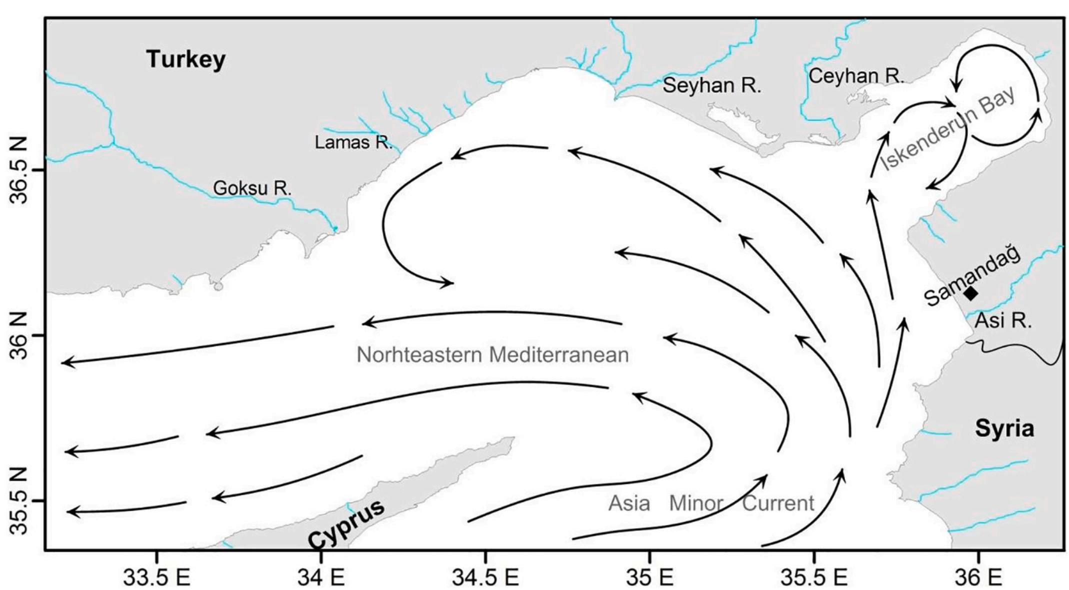


**Fig. 1.** Study area with currents (modified from Collins and Banner, 1979).

**Table 1**
Descriptive information of sampled fish species (habitat, feeding habit, trophic level, fishing vessel, sample size) and morphological statistics (mean ± standard deviation).

| Species | Habitat | Feed on | Trophic level | Fishing vessel | Location | # of fish | Total length (cm) | Weight (g) | GIT weight (g) | Gill weight (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Mullus barbatus* | Demersal | Small benthic crustaceans, worms and mollusks | 3.10 | Trawling | Iskenderun | 20 | 29.37 ± 5.57 | 13.65 ± 0.90 | 0.99 ± 0.32 | 0.87 ± 0.24 |
| | | | | Trawling | Samandağ | 23 | 32.69 ± 9.97 | 13.83 ± 2.62 | 0.90 ± 0.27 | 1.00 ± 0.19 |
| *Mullus surmuletus* | Demersal | Shrimps and amphipods, polychaetes, mollusks, and benthic fish | 3.5 | Trawling | Iskenderun | 21 | 25.25 ± 3.59 | 24.07 ± 5.06 | 0.6 ± 0.28 | 0.75 ± 0.28 |
| | | | | Trawling | Samandağ | 20 | 20.00 ± 3.77 | 12.05 ± 0.94 | 0.85 ± 0.22 | 0.60 ± 0.23 |
| *Saurida undosquamis* | Reef-associated | Fishes (anchovy and red mullet *Mullus surmuletus*), crustaceans | 4.5 | Trawling | Iskenderun | 20 | 49.54 ± 16.33 | 18.88 ± 2.16 | 3.21 ± 1.71 | 0.94 ± 0.43 |
| | | | | Trawling | Samandağ | 19 | 44.70 ± 19.29 | 18.02 ± 4.47 | 1.23 ± 0.77 | 0.98 ± 0.35 |
| *Mugil cephalus* | Benthopelagic | On detritus, micro-algae and benthic organisms | 2.5 | Purse seine fishing | Iskenderun | 10 | 145.67 ± 19.54 | 25.95 ± 1.06 | 6.01 ± 1.07 | 3.53 ± 1.00 |
| | | | | Purse seine fishing | Samandağ | 10 | 205 ± 10.24 | 29.7 ± 0.54 | 9.68 ± 3.12 | 5.60 ± 1.46 |

petri dishes until microscopic examination.

When any identifiable prey was detected in the GIT, it was recorded and analyzed in the same manner as described (Aytan et al., 2021). Identified preys were analyzed to test transfer of microplastic particles from prey to predator. When the prey found in the GIT was not suitable for the species identification, it was degraded within the GIT content.

### 2.4. Microscopic examination

Filters were examined under Olympus CX 41 microscope with an attached Olympus DP 20 digital camera. Color, type, number of MPs, size of estimated MP were recorded. When any interesting microplastic particle was detected, pictures of MPs were taken, placed into glass tube and set aside for Fourier transform infrared (FTIR) analysis.

### 2.5. Fourier transform infrared (FTIR) spectroscopy

In this study, Fourier transform infrared spectroscopy (FTIR) was employed to detect the origin of extracted microplastics. FTIR analysis was carried out on a SHIMADZU QATR10 FTIR spectrophotometer equipped with single reflection attenuated total reflectance (ATR) accessory. The spectrum range was 4000–400 $cm^{-1}$ and a resolution of 4.0 $cm^{-1}$ with 32 scans for each measurement. The polymer type identification was done by comparing absorbance spectra to reference libraries of SHIMADZU library.

### 2.6. Contamination prevention

Sampling, digestion, microscopic examination steps were carried out at closed laboratories with restricted access to prevent airborne contamination (Bessa et al., 2019). All doors and windows were kept closed during analysis (Torre et al., 2016). The laboratory surface and all the equipment used including glass beakers, digestion equipment were cleaned with pure water before and after each dissection procedure. To prevent contamination, glass beakers were covered with tin foil until the sample placement (Bessa et al., 2019). Laboratory personnel always wore gloves and cotton aprons during analysis. Also, wet filters in two replicates were placed into petri dishes during the digestion and microscopic examination for quality control. Blank filters were examined for the microplastic presence at the microscope. No microplastic was observed at the blank filters.

### 2.7. Data analysis

Normality of the data was validated with Kolmogorov-Smirnov and Shapiro-Wilk test. Then, Pearson correlation analysis with 0.05 significance level was employed to test relationship between fish weight, fish length and MP abundance. To investigate the differences in the MP abundance depending on locations and organs one way ANOVA was used. Finally, Tukey test was performed to investigate the differences among species. Statistical analysis was conducted by SPSS 13 and visualization of data was performed by Grapher.

## 3. Results

A total of 153 fish specimen were examined in terms of MPs abundance in the GIT and gill. Information regarding the habitat, feeding habit, trophic level of studied species were obtained from FishBase (Froese and Pauly, 2021) and given in Table 1. MP existence was specified in the GIT and gill of all examined individuals and some examples of extracted MPs were given in Fig. 2. Quantities of isolated MPs obtained from Iskenderun and Samandağ regions were given in Table 2. Majority of the extracted particles were fibers (95%) and followed by fragments (4%). Among all individuals, mean MP abundance in the GIT and gill was found as 4.22 MPs $fish^{-1}$ and 2.70 MPs $fish^{-1}$ in Iskenderun and 11.25 MPs $fish^{-1}$ and 4.66 MPs $fish^{-1}$ in Samandağ, respectively. Statistically significant difference was detected in the GIT of *M. cephalus* and gill of *M. barbatus* and *M. surmuletus* depending on locations ($p < 0.05$).

During digestion procedure, 2 unidentifiable prey and 2 *M. surmuletus* were found in the stomach of *S. undosquamis* sampled from Samandağ station. Unidentified preys were digested with the GIT content. On the other hand, GIT of prey *M. surmuletus*' were degraded and 5 and 1 fiber particles were extracted from each one of them.

Mean MP concentration in the GIT of *M. barbatus*, *M. surmuletus*, *S. undosquamis* and *M. cephalus* was determined as 3.22 MPs $fish^{-1}$, 7.56 MPs $fish^{-1}$, 3.57 MPs $fish^{-1}$ and 26.15 MPs $fish^{-1}$, respectively. The same values were estimated as 3.54 MPs $fish^{-1}$, 4.65 MPs $fish^{-1}$, 2.70 MPs $fish^{-1}$ and 3.85 MPs $fish^{-1}$ in gill. The highest MPs abundance was observed in the GIT of *M. cephalus* sampled from Samandağ. The lowest MP abundance was observed in the gill of *M. barbatus* sampled from Iskenderun Bay (Table 2). Mean MP concentration in the GIT was found to be significantly different among all species in Samandağ ($p < 0.05$). Similar variation was only valid for GIT of *M. barbatus* and *M. surmuletus* in Iskenderun ($p < 0.05$). On the other hand, there were no statistically significant differences detected in the mean MPs abundance in gills at both locations ($p > 0.05$).

MP abundance in the GIT was higher than the gill for all species (Table 2). But, statistically significant difference was valid for *M. cephalus* extracted from Samandağ and *M. surmuletus* extracted from

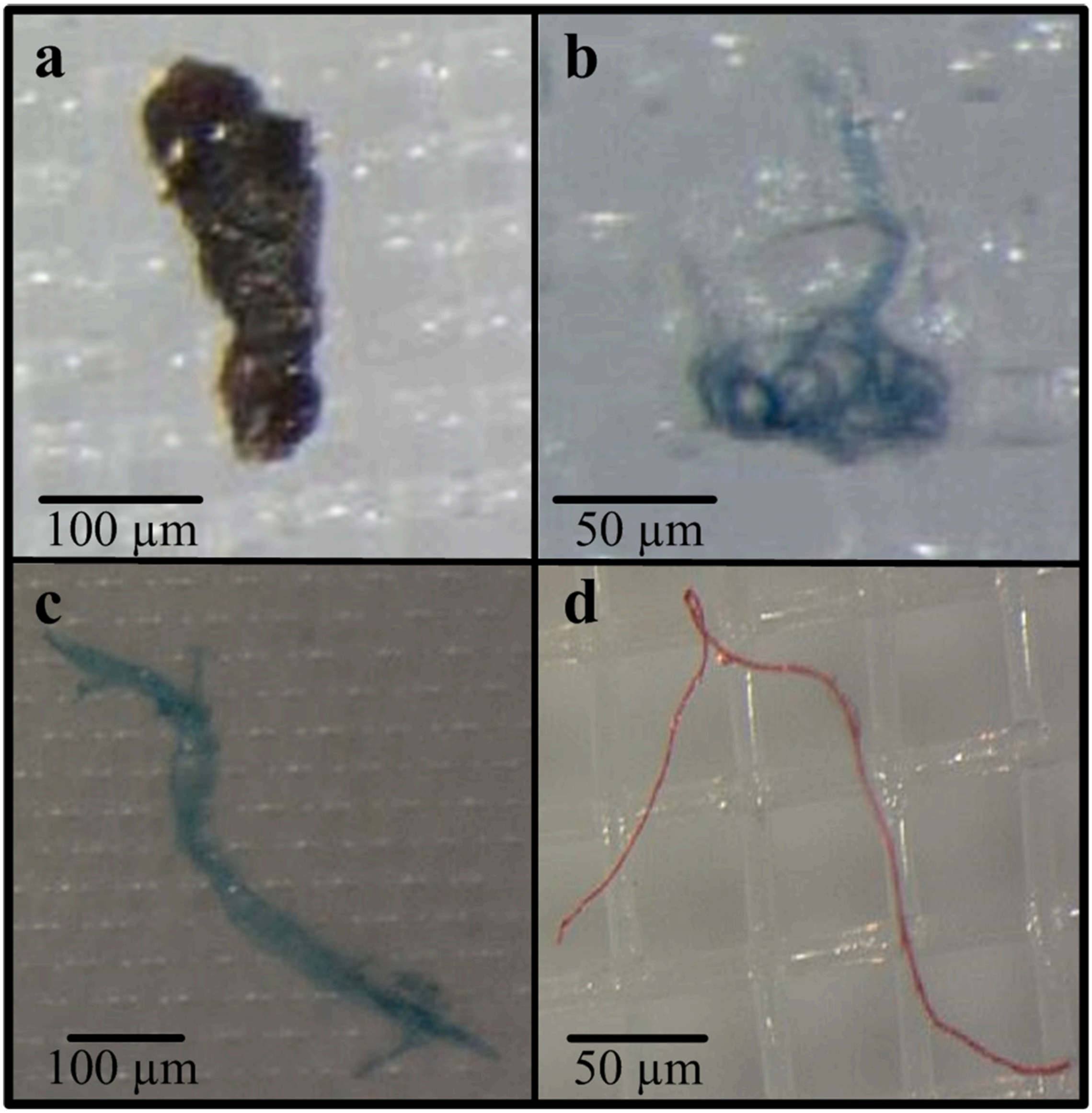


**Fig. 2.** Examples of extracted MPs from *M. barbatus*, *M. surmuletus*, *S. undosquamis*, *M. cephalus*.

**Table 2**
Microplastic abundance in the examined organs (GIT and gill).

| Species | Location | # of sample | GIT | | | | | | Gill | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Fiber | | Fragment | | Mean number of MPs per fish | Frequency of occurrence (%) | Fiber | | Fragment | | Mean number of MPs per fish | Frequency of occurrence (%) |
| | | | n | % | n | % | | | n | % | n | % | | |
| *Mullus barbatus* | Iskenderun | 20 | 57 | 100 | 0 | 0 | 2.9 ± 2.7 (3.2) | 66 | 37 | 97 | 2 | 3 | 1.9 ± 1.7 (2.8) | 71 |
| | Samandağ | 23 | 81 | 98 | 2 | 2 | 3.6 ± 2.8 (4.2) | 85 | 114 | 97 | 4 | 3 | 5.1 ± 4.7 (5.9) | 85 |
| *Mullus surmuletus* | Iskenderun | 21 | 111 | 96 | 5 | 4 | 5.5 ± 4.5 (6.1) | 90 | 66 | 99 | 1 | 1 | 3.2 ± 1.8 (3.4) | 95 |
| | Samandağ | 20 | 184 | 96 | 8 | 4 | 9.6 ± 6.5 | 100 | 112 | 92 | 10 | 8 | 6.1 ± 6.0 (7.2) | 85 |
| *Saurida undosquamis* | Iskenderun | 20 | 65 | 96 | 3 | 4 | 3.4 ± 2.7 (3.6) | 90 | 45 | 88 | 6 | 12 | 2.6 ± 2.8 (3.4) | 75 |
| | Samandağ | 19 | 68 | 96 | 3 | 4 | 3.7 ± 2.2 (3.9) | 94 | 50 | 93 | 4 | 7 | 2.8 ± 2.8 (4.1) | 68 |
| *Mugil cephalus* | Iskenderun | 10 | 58 | 98 | 1 | 2 | 5.9 ± 3.2 | 100 | 34 | 97 | 1 | 3 | 3.5 ± 1.9 (3.9) | 90 |
| | Samandağ | 10 | 426 | 92 | 38 | 8 | 46.4 ± 11.9 | 100 | 40 | 95 | 2 | 5 | 4.2 ± 2.4 (4.7) | 90 |

Number in the brackets represents the mean MPs abundance in the positive samples.

Iskenderun Bay ($p < 0.05$).

A negative strong correlation was observed between body weight and MPs abundance in the gill of *M. barbatus* ($r = -0.33$, $p < 0.05$). Similarly, MPs abundance in the gill *of M. barbatus* and body length was found to be correlated ($r = -0.47$; $p < 0.01$). Another finding was a positive and strong relationship between body weight and MPs abundance in GIT *of M. cephalus* ($r = 0.64$, $p < 0.01$). Lastly, body length and MPs abundance in GIT of *M. cephalus* was found to be correlated with each other ($r = 0.64$; $p < 0.01$).

Among all extracted MPs, black was the most dominant color (53%)

followed by blue (17%) white/transparent (12%), red (11%), green (4%), brown (2%) and orange (<1%). Color distribution was varied depending on species, location and organs (Fig. 3).

The average size of MPs extracted from GITs was 1.33 ± 1.31 mm and extracted from gill was 1.2 ± 1.3 mm. Majority of the extracted MPs were within the range of 0.5–2.5 mm (Fig. 4).

Morphologically different plastic-like particles were subjected to FTIR analysis. Among all, 34% of examined particles was detected as Polyethylene (PE), Polyethylene derivatives (PET); while, 5% of examined particles was detected as polyamide (Fig. 5).

In general, stomachs of the studied species were filled with the remaining parts of prey crustaceans and small fish. However, stomach content of *M. cephalus* samples ($n = 10$) obtained from Samandağ region were filled with sand particles (Table 1).

## 4. Discussion

After the establishment of The Marine Strategy Framework Directive, studies investigating the MP occurrence in the aquatic organisms have been extensively conducted (Habib and Tieaman, 2021). Previous studies showed that Turkey, Egypt and Italy coast of Mediterranean Sea are considered as the top emitters of plastic due to intense discharges coming from major rivers and large urban areas (Papadimitriu and Allinson, 2022). Therefore, examination of microplastic occurrence in these hot spot regions is important to create necessary legislations and protection strategies. This study, as far as we know, is the first report examining the MP abundance in the gill of four different species from the northeastern Mediterranean Sea.

Previous studies reported the frequency of occurrence in the GIT of *M. barbatus* as 14.3% in the Central Mediterranean (Capillo et al., 2020), 18% (Bellas et al., 2016), 20% in the Italian coast of the Mediterranean (Giani et al., 2019), 24% (Aytan et al., 2021), 25% (Atamanalp et al., 2021) in Black Sea, 32% in Ionian Sea (Digka et al., 2018), 42% (Güven et al., 2017), 50% (Rodríguez-Romeu et al., 2020), 60% (Gündoğdu et al., 2020) in northeastern Mediterranean Sea and 61% in the Marmara Sea (Gündoğdu et al., 2020). MP ingestion frequency of *M. surmuletus* was reported as 27% in western Mediterranean Sea (Alomar et al., 2016), 35% in northeastern Mediterranean Sea (Güven et al., 2017), 56% in Aegean Sea (Gündoğdu et al., 2020). MP occurrence rate in the GIT of *S. undosquamis* was reported as 36% in northeastern Mediterranean Sea (Güven et al., 2017). The frequency of occurrence in the GIT of *M. cephalus* was reported as 60% in Hong Kong (Cheung et al., 2018), 42% in Mexico (Borges-Ramírez et al., 2020), 64% in Sydney Harbour (Halstead et al., 2018), 73% in South Africa (Naidoo et al., 2016), 100% in China (Jabeen et al., 2017).

In this study, mean MPs abundance in GIT of *M. barbatus* was determined as 3.22 MPs fish$^{-1}$ which is higher than the previously conducted studies in the Mediterranean Sea (Avio et al., 2015; Bellas et al., 2016; Güven et al., 2017; Digka et al., 2018; Giani et al., 2019; Gündoğdu et al., 2020; Capillo et al., 2020; Rodríguez-Romeu et al., 2020) and in the Black Sea (Atamanalp et al., 2021; Aytan et al., 2021). Mean MPs abundance in the GIT of *M. surmuletus* (5.52 MPs fish$^{-1}$ in Iskenderun and 9.60 MPs fish$^{-1}$ in Samandağ) was significantly higher than previous reports from the Mediterranean Sea (Alomar et al., 2016; Güven et al., 2017; Gündoğdu et al., 2020). Lower MP abundance in the GIT of *S. undosquamis* than this study was reported in northeastern Mediterranean Sea (Güven et al., 2017). For *M. cephalus* extracted from Iskenderun Bay, lower MPs abundance was reported in China (Jabeen et al., 2017; Borges-Ramírez et al., 2020) and South Africa (Naidoo et al., 2016); whereas, comparable results were reported by Halstead et al. (2018), Saha et al. (2021), Guilhermino et al. (2021) (Table 3). Differently, MPs abundance in the GIT of *M. cephalus* extracted from Samandağ region was extremely higher than other species and previous studies (Table 3). *M. cephalus* often enter the estuaries and rivers and accommodate on sand or mud-bottom. Recent study showed the formation of plastic deposition pits at the seabed near the Asi River estuary (Yılmaz et al., 2022). Higher abundance in the GIT may be related with the recent feed on the waste deposition pits; since, the stomach of the fish was filled with sand (Table 1). Similarly, Güven et al. (2017) concluded that the presence of MPs in GIT indicates the recent ingestion of MPs rather than bioaccumulation potential.

MPs may attach to gills during water flow. Abbasi et al. (2018) reported the mean MPs abundance in the gill of different fish as 3.81 (±2.19) MPs fish$^{-1}$ in the Persian Gulf. Capillo et al. (2020) isolated fibers from the gills of *Trigla lyra* with an average of 0.19 MPs fish$^{-1}$ in central Mediterranean Sea. Koongolla et al. (2020) reported the mean

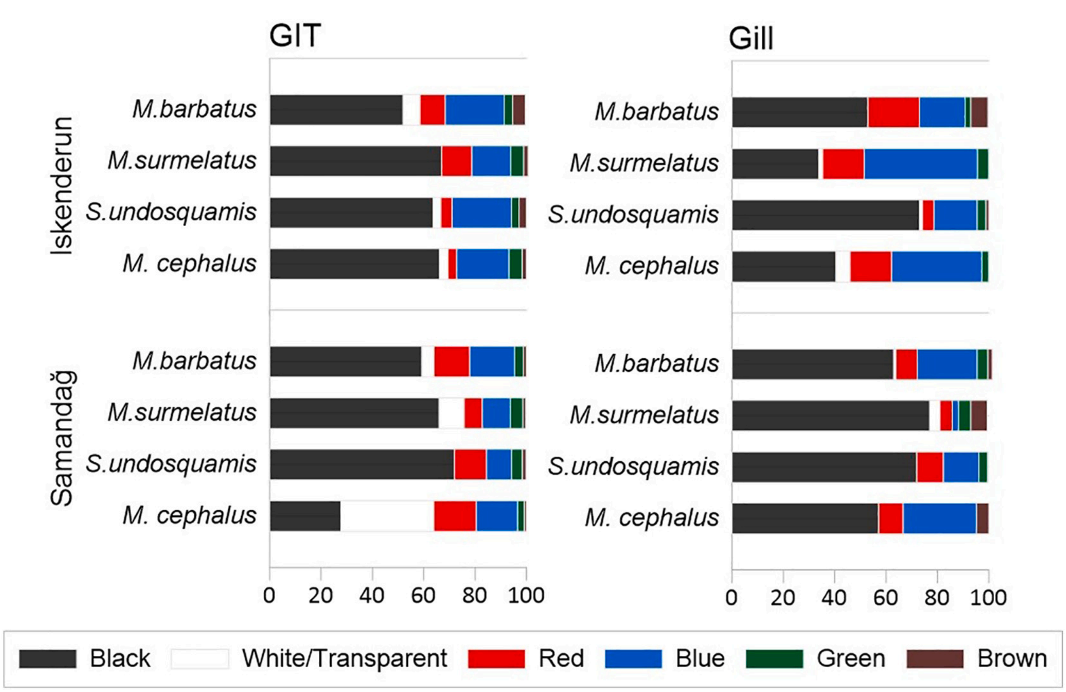


**Fig. 3.** Percentage of all extracted microplastics categorized by color.

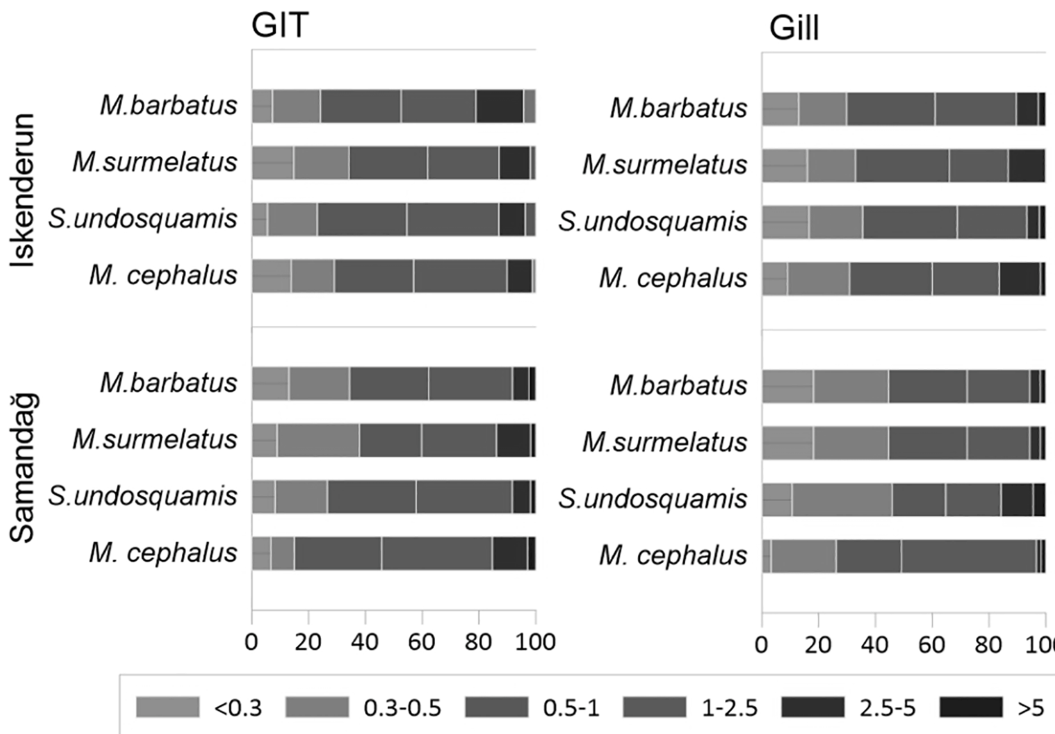


**Fig. 4.** Size range of extracted microplastics (in mm).

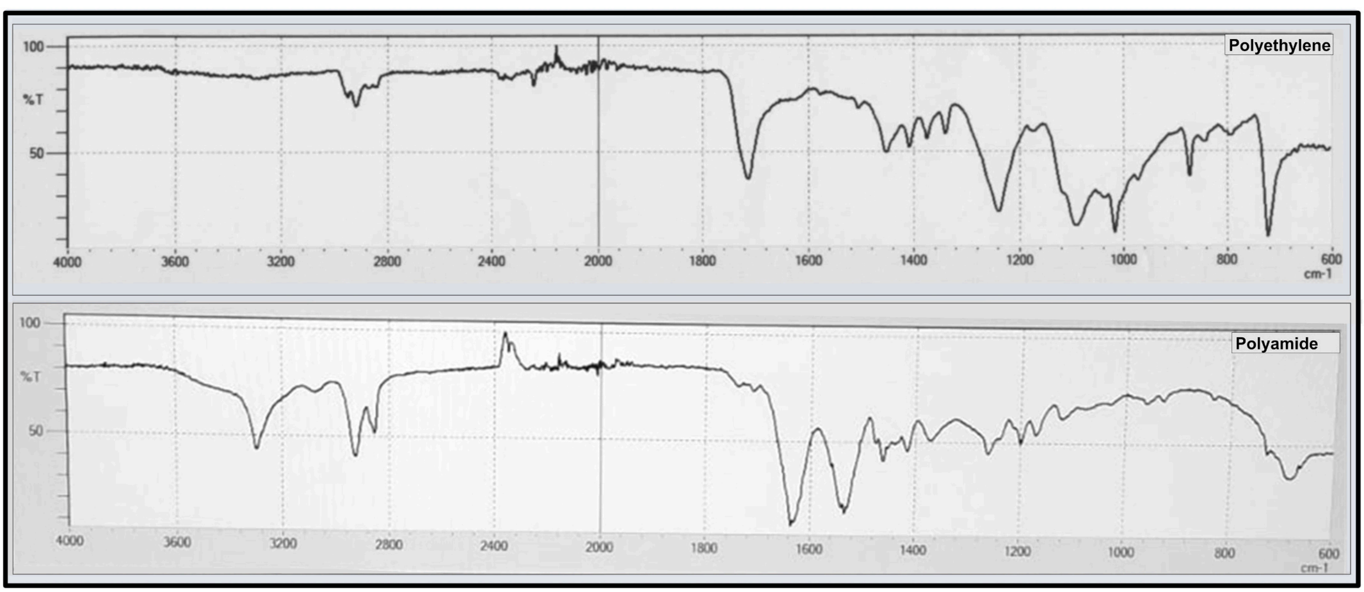


**Fig. 5.** Representative FTIR spectra for major identified polymers.

MP abundance in the gill of 24 different fish as 0.16 (±0.15) MPs fish$^{-1}$ in South China. Atamanalp et al. (2021) reported the mean MP abundance in the gill of *M. barbatus* as 0.62 MPs fish$^{-1}$ in Black Sea. Results obtained in this study were higher than Koongolla et al. (2020), Capillo et al. (2020), Atamanalp et al. (2021); whereas, it is comparable to Abbasi et al. (2018).

The heterogeneity of the results in the previous studies regarding the MPs occurrence in GIT and gill of selected species could be due to differeces in the employed analytical methods, differences in pollution levels, differences in major anthropogenic activities takeing care of at the locations, changes in hydrodynamic circulation or sampling time (Pellini et al., 2018; Digka et al., 2018; Gündoğdu et al., 2020; D. Zhang et al., 2020).

MPs may be ingested actively by mistaken for food or they may be ingested passively by either foraging or prey (Roch et al., 2020). In this way, trophic transfer of MPs was reported in both marine environments and laboratory experiments (Costa et al., 2020; da Costa Araújo and Malafaia, 2021). In this study, fibers found in the prey *M. surmuletus* indicate the transfer of MPs to higher trophic levels. A significant negative correlation was observed between MPs abundance in GIT and trophic level ($r = -0.403$, $p < 0.01$). Similar to our result, Walkinshaw et al. (2020) concluded that lower trophic levels were at higher MPs

**Table 3**
Recent literature indicating abundance and predominant type of microplastic in studies species.

| Species | Collection site | GIT | Gill | Dominant color | Predominant type | Reference |
|---|---|---|---|---|---|---|
| *Mullus barbatus* | NE Mediterranean Sea | 1.59 | – | Blue | Fiber | Güven et al., 2017 |
| *Mullus barbatus* | Black Sea | 0.76 | 0.62 | Black | Fiber | Atamanalp et al., 2021 |
| *Mullus barbatus* | Aegean | 1.10 | – | Black | Fiber | Gündoğdu et al., 2020 |
| *Mullus barbatus* | NE Mediterranean Sea | 1.3 | – | Black | Fiber | Gündoğdu et al., 2020 |
| *Mullus barbatus* | North Tyrrhenian Sea, Adriatic Sea, Ionian Sea | 1.08 | – | Blue | Fiber | Giani et al., 2019 |
| *Mullus barbatus* | Black Sea | 0.38 | – | Black | Fiber | Aytan et al., 2021 |
| *Mullus barbatus* | Central Mediterranean Sea | 0.3 | – | Black | Fiber | Capillo et al., 2020 |
| *Mullus barbatus* | Ionian Sea | 0.5 ± 0.2 | – | Blue | Fragment | Digka et al., 2018 |
| *Mullus barbatus* | Mediterranean Sea | 1.9 ± 1.29 | – | Black | Fiber | Bellas et al., 2016 |
| *Mullus barbatus* | Adriatic Sea | 1.57 ± 0.78 | – | – | Fragment | Avio et al., 2015 |
| *Mullus barbatus* | NW Mediterranean Sea | 1.48 ± 1.98 | – | – | Fiber | Rodríguez-Romeu et al., 2020 |
| *Mullus surmuletus* | Western Mediterranean Sea | 0.68 ± 0.10 | – | Blue | Filament | Alomar et al., 2016 |
| *Mullus surmuletus* | Mediterranean Sea | 1.52 | – | Blue | Fiber | Güven et al., 2017 |
| *Mullus surmuletus* | Aegean Sea | 1.30 | – | Black | Fiber | Gündoğdu et al., 2020 |
| *Saurida undosquamis* | Mediterranean Sea | 1.51 | – | Blue | Fiber | Güven et al., 2017 |
| *Mugil cephalus* | Guangdong, South China | 5.2 | – | White | Fiber | C. Zhang et al., 2020 |
| *Mugil cephalus* | Hong Kong | 4.3 | – | Green | Fiber | Cheung et al., 2018 |
| *Mugil cephalus* | Guangdong, China | 5.2 | – | – | – | D. Zhang et al., 2020 |
| *Mugil cephalus* | China | 3.7 ± 1.0 | – | Transparent | Fiber | Jabeen et al., 2017 |
| *Mugil cephalus* | Mexico | 1.2 | – | Black | Fragment | Borges-Ramírez et al., 2020 |
| *Mugil cephalus* | China | 10 ± 9 | – | Black | Fiber | Guilhermino et al., 2021 |
| *Mugil cephalus* | Sydney Harbour | 4.6 (±1.2) | – | – | Fiber | Halstead et al., 2018 |
| *Mugil cephalus* | South Africa | 3.8 | – | White and clear | Fiber | Naidoo et al., 2016 |

ingestion risk. On the other hand, Zhang et al. (2019) reported a positive correlation between trophic level and MP abundance in the East China Sea.

When all species examined in this study were analyzed regardless of station, a strong correlation between fish weight and MP abundance in GIT ($r = 0.71$, $p < 0.01$), fish length and MP abundance in GIT ($r = 0.10$, $p < 0.01$) and fish length and MP abundance in gill ($r = -0.24$, $p < 0.01$) were detected. Similar correlation was detected by Atamanalp et al. (2021); whereas; no correlation was reported by Güven et al. (2017). It should be noted that much larger sample size was required for more accurate results to test the relationship between fish size/weight and MPs abundance which was not the objective of this study.

Even though variation in the MP abundance depend on the organs was not significant for all studies species, MPs abundance in the GIT was higher than gill. This might be related with the different spatial constraints of organs considering the MPs size and distribution.

MPs abundance in GIT and feeding strategy of species are highly related with each other. For instance, Digka et al. (2018) found that filter feeder species have the highest MP ingesting frequency. Similarly, Gündoğdu et al. (2020) reported the higher MPs ingestion possibility of planktivorous fish than piscivorous fish. Among the studied species herein, *M. barbatus*, *M. surmuletus*, *S. undosquamis* are carnivorous species and *M. cephalus* were omnivorous. Highest MP abundance in the GIT was found in plankton feeder *M. cephalus* and lowest abundance was found in the carnivore *S. undosquamis*.

It is known that some polymer types like PE tend to stay in water column; whereas, some like PET more likely sink to the sea bottom (Digka et al., 2018). For that reason, MP ingestion rate and habitat of fish were reported to be correlated (D. Zhang et al., 2020). Anastasopoulou et al. (2018) reported that MP were more frequently found in the demersal fish in S. Adriatic Sea; while, it is in the pelagic fish in NE Ionian Sea. Similarly, some studies reported higher MP ingestion frequency in pelagic fish (Güven et al., 2017; Aytan et al., 2021), whereas, others reported higher in demersal fish (Gündoğdu et al., 2020; Koongolla et al., 2020). Alternatively, Bessa et al. (2018) reported higher MP abundance in benthopelagic than demersal species. In this study, MP abundance in GIT was, from the largest to the smallest, benthopelagic *M. cephalus*, demersal *M. surmuletus*, demersal *M. barbatus* and reef-associate *S. undosquamis*, respectively.

As mentioned previously, *M. barbatus*, *M. surmuletus* and *M. cephalus* were accommodate on the sea bottom. While feeding, they expell the sediment through the gills (Labropoulou and Eleftheriou, 1997); whereas, *S. undosquamis* was reef-associated and feed on small fish. In this study, MPs abundance in the gill did not change depending on species ($p > 0.05$) but varied depending on locations ($p < 0.05$). Therefore, MP abundance in the gill may be related with MP pollution in the sampling location rather than feeding habit or habitat of fish.

In agreement with the previous studies, fibers were the major plastics extracted from the fish (95%) (Table 3). Previous study concluded that fiber type microplastics were more commonly found in populated areas which is under the influence of anthropogenic activities; whereas, fragment type plastics were found in protected areas with no wastewater input (Alomar et al., 2016). Major sources of fiber were reported as fragmentation from fishing nets (Andrady, 2015; Koongolla et al., 2020), textile industry (Browne et al., 2011; Mancuso et al., 2019), washing machines (Hartline et al., 2016).

In terms of color, black was the most dominant color in extracted MPs which is coherent to the previous studies (Gündoğdu et al., 2020; Atamanalp et al., 2021; Aytan et al., 2021; Capillo et al., 2020). As a result of decrease in light transmittance in benthic environment, demersal fish may accidently ingest black MPs (Koongolla et al., 2020). In addition, Atamanalp et al. (2021) reported that younger fish prefer black color more; since, this color is more similar to their food (Ferreira et al., 2020). Different from general picture, white/transparent color was the most abundant color extracted from the GIT of *M. cephalus* in Samandağ (Fig. 3). Most plankton and algae species are transparent and white in color which leads to the ingestion of these plastic particles accidentally for food (Wang et al., 2020). Previous studies also reported white/transparent color (Naidoo et al., 2016; C. Zhang et al., 2020) and blue color (Güven et al., 2017; Giani et al., 2019) as dominant color.

Small microplastics (<1 mm) constitutes the majority of extracted MPs regardless of the species, location or organ (Fig. 4). These particles have a strong capacity for hydrophobic organic pollutants which makes them a pollutant vector for aquatic organisms (Zhu et al., 2018). Similar situation was also reported by previous studies (C. Zhang et al., 2020; D. Zhang et al., 2020; Atamanalp et al., 2021).

In this study, *M. barbatus*, *M. surmuletus* and *M. cephalus* were used as indicator species for MP pollution in the seabed; since, they were suggested as bioindicator organisms for MP ingestion by previous research (Gökdağ, 2017; C. Zhang et al., 2020). Results obtained in this study showed higher MPs abundance in the organs at Samandağ region (Table 2). Plastic pollution level in the Samandağ coastal was reported to be higher than Iskenderun Bay (Yılmaz et al., 2022). As a result, this study showed that as the plastic amount in marine debris increases, MPs ingestion by fish also increases.

Major polymer types detected (PE, PET) in this study were similar to those conducted in Mediterranean Sea (Güven et al., 2017; Capillo et al., 2020), Red Sea (Sayed et al., 2021), Black Sea (Atamanalp et al., 2021) and China (Koongolla et al., 2020). As a result of global production of polyethylene (Digka et al., 2018), polyethylene derivatives was determined as the most commonly found polymer in the fish. Major sources of PE were reported as plastic bags and bottles (Cózar et al., 2017; Suaria et al., 2016).

## 5. Conclusion

In this study, *Mullus barbatus*, *Mullus surmuletus*, *Saurida undosquamis*, *Mugil cephalus*, were used as bioindicator organisms which reflect the microplastic pollution status in the sediment of two different coastal areas of the northeastern Mediterranean. The highest MP levels in the GIT and gill of fish was determined at Asi River estuary (Samandağ). Results showed that MPs abundance in the gill is mostly depended on microplastic pollution at the surrounding environment; however, abundance in the GIT is affected by multiple factors like habitat, feeding strategy, color. Most of the extracted MP was fiber which indicates the poor water quality status of the region. Polyethylene derivatives were the common polymer type which is compatible with the global production. Results obtained in this study contribute to the knowledge of microplastic contamination level in the sediment and usage of these species as bioindicators. In addition, legislations for the protection marine environments from microplastic pollution are necessary considering high ingestion rates reported in this study.

## CRediT authorship contribution statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors (Ece KILIÇ and Nebil YÜCEL). The first draft of the manuscript was written by Ece KILIÇ and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Declaration of competing interest

This manuscript is not under consideration for publication elsewhere, and its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

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