



## Investigation of Microplastic Accumulation in Horse Mackerel (*Trachurus mediterraneus*) Caught in the Black Sea

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**Abstract:** The marine ecosystem is prone to pollution exposure due to a number of factors. Microplastic (MP) pollution has been a severe issue recently; however, studies on marine organisms are limited. The abundance and composition of MPs in highly consumed horse mackerel caught from the Turkish coast of the Black Sea were investigated here. A total of 27 MPs were detected in 121 horse mackerel (*Trachurus mediterraneus*). The mean of MPs per fish was calculated as  $0.22 \pm 0.14$ . While polyethylene was the most prevalent type, fiber, with a length range of 500 to 1000  $\mu\text{m}$  (33%), was the most common form. These findings suggest that more study is required to determine the magnitude of MP contamination in the Black Sea.

**Keywords:** Fish, microplastic, black sea, horse mackerel.

## Karadeniz'de Yakalanan İstavritte (*Trachurus mediterraneus*) Mikroplastik Birikiminin Belirlenmesi

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**Öz:** Denizel ortamlar, özellikle nehirlerin taşımış olduğu kirleticilere maruz kalan hassas alanlardır. Son yıllarda mikroplastik kirliliği güncel bir konu olmasına rağmen denizel organizmalar üzerine yapılan çalışmalar sınırlıdır. Bu çalışmada Karadeniz'in Türkiye kıyılarından avlanan ve yüksek oranda tüketilen istavritteki mikroplastiklerin bolluğu ve kompozisyonu araştırıldı. Analiz edilen 121 adet istavritte toplam 27 adet mikroplastik tespit edildi. Sindirilen mikroplastiklerin balık başına ortalaması  $0,22 \pm 0,14$  adet olarak hesaplandı. Belirlenen mikroplastiklerde en baskın şekil olarak fiber (%66) iken, boy olarak ise 500-1000  $\mu\text{m}$  (%33) arasında dağılım gösterdiği saptandı. Bu sonuçlar, Karadeniz'deki MP kontaminasyonunun boyutunu değerlendirmek için daha fazla araştırmaya ihtiyaç olduğunu göstermektedir.

**Anahtar kelimeler:** Balık, mikroplastik, karadeniz, istavrit.

## INTRODUCTION

Plastic manufacturing has risen recently as a result of the increased demand for plastic items, which is a significant contributor to ecological issues (Rebelein et al., 2021). Although there has been more research on the impacts of large-sized (macro) plastics (Galarpe et al., 2021; Vriend et al., 2020), the effects of smaller-sized plastics have frequently been disregarded (Md Amin et al., 2020; Onay et al., 2023). Microplastic (MP) is defined as plastic particles

smaller than 5000  $\mu\text{m}$  and has two main sources (Thiele et al., 2021). While those produced in microscopic size are defined as primary MP sources, small plastic parts formed from the fragmentation of larger plastic pieces constitute secondary MP sources (Cole et al., 2011).

Despite being widespread in marine ecosystems, MPs are now thought to be one of the leading causes of environmental stress (Mallik et al., 2021; Masud et al., 2022). Among the species in the entire aquatic ecosystem, fish is just one of the organisms affected by MP (Mallik et

al., 2021). Since MPs have a similar size range as the plankton species that make up the fish food, MPs in water can be easily ingested by fish (Ding et al., 2018). However, when MP is swallowed and builds up in various fish tissues, it can have a variety of impacts, including biological system abnormalities, mortality, along with other adverse effects (Bhuyan, 2022).

Six nations border the semi-enclosed Black Sea, including Bulgaria, Georgia, Romania, Russia, Ukraine, and Türkiye (Apaydın et al., 2022). Although it receives water from significant rivers like the Danube, Dnieper, and Don, the Black Sea is susceptible primarily to anthropogenic pollution from rivers, including those from industry, marine, agriculture, household, tourism, and fishing activities (Bat et al. 2022; Eryaşar et al., 2022). Heavy metal concentrations in water, sediment, and marine life were mainly measured as part of pollution investigations in the Black Sea (Alkan et al., 2012; Bat et al., 2007; Gedik and Ozturk, 2018; Karsli, 2021; Mutlu, 2021a, 2021b; Özşeker et al., 2022a, 2022b; Polat and Akkan, 2016). Due to limited studies, microplastic contamination has not been as common in the Black Sea in recent years as in other international waters (Eryaşar et al., 2022; Gedik & Eryaşar, 2020; Gedik & Gozler, 2022; Terzi et al., 2022). The studies generally investigated the abundance of MP in the water column, sediment and mussels. However, in terms of fish species, only Eryaşar et

al. (2022) investigated the abundance of MP in the digestive tract of anchovy (*Engraulis encrasicolus*), red mullet (*Mullus barbatus*), and whiting (*Merlangius merlangus*) sampled along the Black Sea coast of Türkiye. Identifying MPs for additional significant commercial fish species is critical in this regard.

Horse mackerel is the most caught fish species in Türkiye after anchovy and sprat (BSGM, 2021). Horse mackerel (*Trachurus mediterraneus*), which plays an essential role in commercial fisheries, is widely distributed in the Black Sea and feeds on copepods, decapods, fish larvae, small fish, and cephalopods (Yankova et al., 2008). For the horse mackerel, one of the most often caught species along Türkiye's Black Sea coast and a species that consumers frequently consume. This study set out to determine the number, distribution, and characterization of MPs.

## MATERIAL AND METHOD

**Fish sampling:** Fish samples were obtained with purse seine fishing activities between August and December 2021 from nine different sampling points on the Black Sea coast of Türkiye (Figure 1). The fish samples' length and weight were measured after they were transported to the lab in aluminum foil with a cold chain (Tablo 1).



Figure 1. Sampling area. Horse mackerel (*Trachurus mediterraneus*) samples obtained from 9 different stations.

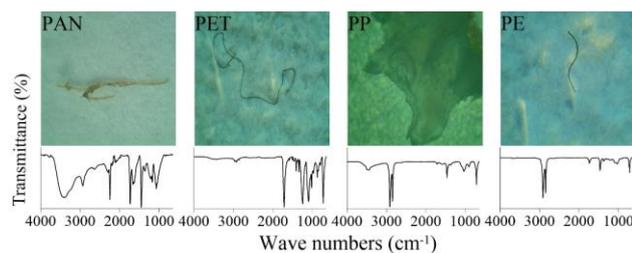
Table 1. Length-weight data and microplastic abundance of horse mackerel (*Trachurus mediterraneus*) sampled from the Black Sea.

Stations'ID	Stations' name	Analyzed Fish (N)	Length (cm)	Fresh weight (g)	Total MPs (#)	MP per fish
1	İğneada	28	13.04±1.38	20.18±7.16	5	0.32±0.14
2	Sakarya	12	11.88±0.65	16.21±2.70	2	0.17±0.00
3	Zonguldak	20	13.32±0.70	20.06±4.10	8	0.40±0.14
4	Sinop	11	12.72±0.89	18.27±3.93	4	0.35±0.21
5	Samsun	10	12.07±0.63	16.13±2.53	2	0.20±0.00
6	Giresun	10	12.34±0.90	17.19±2.09	1	0.10±0.14
7	Trabzon	10	12.72±0.86	17.85±2.91	3	0.30±0.14
8	Rize	10	12.21±1.01	17.24±5.11	1	0.10±0.14
9	Artvin	10	11.99±0.66	15.65±1.89	1	0.10±0.14

MP: microplastic, results were given as average±standard deviation.

**MP extraction:** Avio et al. (2015) and Jabeen et al. (2017)'s method were used to extract MPs. Before dissection of the gastrointestinal tract (GIT), fish were washed with pre-filtered ultrapure water to avoid MP contamination from external sources. After the fish was dissected and the GITs were removed, samples were placed in flasks. Hydrogen peroxide ( $H_2O_2$  30%, Tekkim Co.) (1:10 GIT:  $H_2O_2$ ) was added to the flasks where the samples were stored and covered with aluminum foil, then kept at  $65^\circ C$  to digest. When GIT samples were completely digested, the solution in the beakers was passed through Whatman No 1 filters (Whatman Co.). The filter papers were placed in glass petri dishes, let to air dry, and then kept for microscopic observation (Hidalgo-Ruz et al., 2012).

**MP observation:** After drying, the filters were examined under a stereomicroscope (Kyowa SE-L, Japan) (Hidalgo-Ruz et al., 2012). With a needle, all particles presumed to contain MP were transferred to a clean filter. Then, the particles were photographed (Figure 2) by the camera on the stereomicroscope, counted, and classified according to their shape (fiber, fragment, and film) after measuring the lengths. Finally, Attenuated Total Reflection-Fourier Transform Infrared Spectrometer (PerkinElmer Spectrum 100, ATR-FTIR) was used to confirm the particles and determine the polymer type (Figure 2). For polymer type identification, the spectral bandwidth was set to  $4000-650\text{ cm}^{-1}$ , and 18 repetitive scans were performed at  $4\text{ cm}^{-1}$  resolution. The data obtained at this stage were compared with the data in the device's library, and only particles with  $>70\%$  similarity were reported as MP (Gedik & Eryaşar, 2020).



**Figure 2.** Stereomicroscope images and ATR-FTIR spectrums of microplastics in the gastrointestinal tract (GIT) of horse mackerel sampled from Black Sea. PAN: polyacrylonitrile, PET: polyethylene terephthalate, PP: polypropylene, PE: polyethylene.

**Quality assurance and control:** All operations in the laboratory were carried out in a controlled environment. A rigorous standard practice and several strict precautions were taken to prevent potential MP contamination during the experimental procedure. During the experiments, cotton lab coats and polymer-free gloves were preferred. All liquids (ultrapure water and  $H_2O_2$ ) were filtered through a filter paper (47 mm diameter,  $1.2\ \mu\text{m}$  pore diameter). In addition, the labware used during the

experiments (aspirator bottle, filtering set, beaker, flask, petri dish, etc.) was washed with filtered water and closed with aluminum foil before use.

Negative control blanks were applied to detect contamination during MP extraction from GIT samples by adding only  $H_2O_2$  to 5 flasks with the samples. The MP abundance of the samples was corrected for the polymer types and numbers detected in the negative controls.

As a positive control, a certain amount and length ( $150-212\ \mu\text{m}$ ) of PE, PP, and PET were prepared as specified in Pehlivan & Gedik (2021, 2022). Then, the muscle tissues between the lateral line and the dorsal fin of the randomly selected fish were removed and placed in the flasks. The regular extraction procedure was applied (three times) by adding  $H_2O_2$  with prepared MPs. The recovery was calculated by counting the added MPs under the microscope. The results were determined as 91%, 94%, and 90% for PE, PP, and PET, respectively.

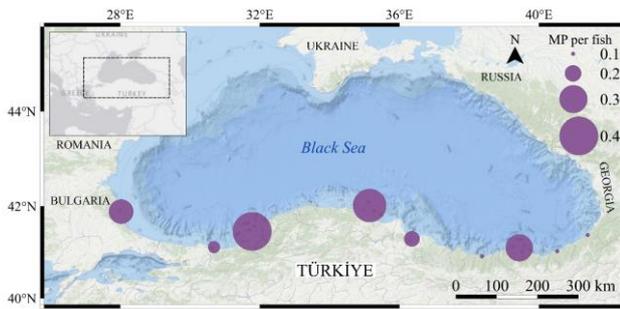
Washed petri dishes filled with filtered ultrapure water (blanks) were placed next to the microscope to determine if there was any contamination from the air during the microscopic examination. If any MP was recorded in the blank samples, the background was adjusted by subtracting the detected amount from the total MP value in the series.

**Data evaluation:** The MP abundance of fish samples is presented as the number of particles per individual. Shapiro-Wilk and Levene tests were used to test the normal distribution of data and homogeneity of variances. Kruskal Wallis-H test was used to determine the differences between stations, and the significance value was set as 0.05. Statistical analyzes of the data were performed using JMP 14.1.0 (SAS Institute Inc.).

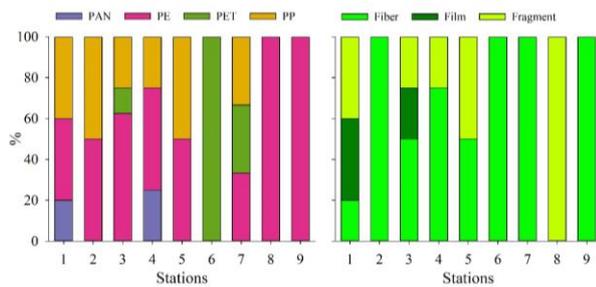
## RESULTS

A total of 121 horse mackerel GITs from 9 different stations along the Black Sea coast of Türkiye were examined, and a total of 27 MPs were determined. While MP abundance was similar at stations 2, 5, 6, 8, 9 and 1,3,4,7, these two station groups were statistically different from each other (Kruskal Wallis-H,  $p < 0.05$ ). The MP abundance varied according to the MP stations detected in the GITs, with the lowest  $0.10 \pm 0.14$  MP and the highest  $0.40 \pm 0.14$  MP per individual, while the mean was calculated to be  $0.22 \pm 0.14$  MP (Table 1; Figure 3).

As a result of the ATR-FTIR examination, the detected MPs were determined to belong to 4 polymer types: PAN: polyacrylonitrile, PET: polyethylene terephthalate, PP: polypropylene, PE: polyethylene. The polymer types are as follows, in order from the most to the least; PE (52%) > PP (30%) > PET (11%) > PAN (7%) (Fig. 3).



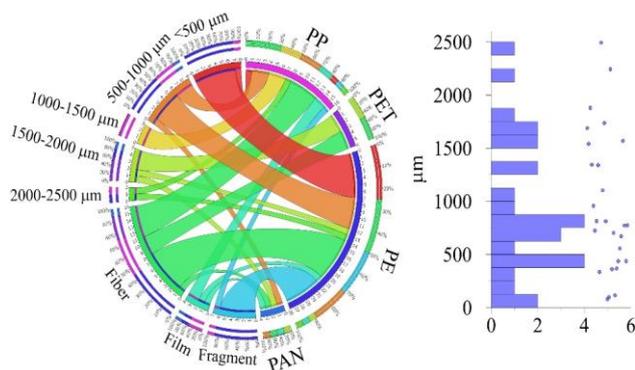
**Figure 3.** Spatial distribution of microplastics in the gastrointestinal tract (GIT) of *Trachurus mediterraneus* sampled from Black Sea.



**Figure 4.** Characterization of microplastics in the gastrointestinal tract (GIT) of *Trachurus mediterraneus* sampled from Black Sea.

The results regarding the characterization, polymer compositions, and morphologies of MPs determined in horse mackerel GITs are presented in Figure 4. The total of 27 MPs obtained from GITs was in 3 different forms, 16 of which were fiber, 4 were film, and 7 were fragments. The MPs detected in horse mackerel GITs at all stations were predominantly fiber (60%). The fiber is followed by the fragment detected at 5 stations (27%) and the film detected at 2 stations (7%) (Figure 3).

The MPs identified in the GITs of the horse mackerels were found to have an average ( $\pm$ standard deviation) length of  $971 \pm 650 \mu\text{m}$ , with sizes ranging from  $102 \mu\text{m}$  to  $2477 \mu\text{m}$  (Figure 5). According to the evaluations, the length group in the range of  $500\text{--}1000 \mu\text{m}$  was the densest group with 33%. The  $102\text{--}500 \mu\text{m}$  group followed this with 30%, the  $1500\text{--}2000 \mu\text{m}$  group with 19%, the  $1000\text{--}1500 \mu\text{m}$  group with 11%, and at last,  $2000\text{--}2500 \mu\text{m}$  with 7%.



**Figure 5.** Polymer compositions and morphologies of microplastics in the gastrointestinal tract (GIT) of *Trachurus mediterraneus* sampled from Black Sea.

## DISCUSSION AND CONCLUSION

This study examined whether MPs were present in the GITs of a horse mackerel heavily caught in the Black Sea, a favorite among locals. The mean number of MPs per fish found was calculated as  $0.22 \pm 0.14$ . Neves et al. (2015), investigating the MP abundance of 26 species of fish on the Portuguese coast, found the average MP number per fish for horse mackerel to be lower ( $0.07 \pm 0.25$ ) in their study than the average value we obtained in our study. However, in another study conducted in Portugal, a higher number of MPs per individual (1.75) was observed (Lopes et al., 2020). Due to the lack of research on MP abundance in horse mackerel along the Black Sea coastlines of Türkiye, fish/MP studies' outcomes were compared at similar sampling sites. In one of these studies, the average MP abundance per fish was determined as 0.15, 0.28, and 0.40 for *Engraulis encrasicolus*, *Merlangius merlangus*, and *Mullus barbatus*, respectively (Eryaşar et al., 2022). It can be concluded that our results are higher when compared to anchovies, fish that inhabits a comparable habitat. This discrepancy is believed to be the result of the diverse study locations and sampling times, as well as the various fish species' observed morphological and feeding patterns (Neves et al., 2015; Peters et al., 2017). In addition, the distributions of MPs in the GITs differed between stations (Figure 2). This difference might be related to the MP abundance in the seawater of the locations where the samples were obtained (Sun et al., 2019).

In the horse mackerel species sampled in the Black Sea, the dominant polymer type was found to be PP and PE. Polymers, which are widely distributed in aquatic ecosystems around the world and are one of the most frequently produced polymers by the plastics industry (Lopes et al., 2020; GESAMP, 2015), have been shown to have a tendency to float on the water surface due to densities that are lower than that of water (Digka et al., 2018). Moreover, this evaluation is in line with the findings of other Black Sea research investigations (Cincinelli et al., 2021; Gedik & Eryaşar, 2020; Gedik & Gözler, 2022; Pojar et al., 2021).

The majority of particles ingested by fish were fiber (60%). The majority of research (Barboza et al., 2020; Eryaşar et al., 2022; Lusher et al., 2013) have also noted that fibers are the most typical MPs found in marine habitats. Yet, Bessa et al. (2018); Compa et al. (2018); Lusher et al. (2013); Kılıç (2022) determined a higher rate of fiber than the current work. Fibers may have been leached into the seas from materials used in the fishing industry (rope, net, and other), sewage discharges, and terrestrial sources (wastewater from washing machines, the textile industry, port industry) (Barboza et al., 2020; Kılıç

et al., 2022). It has been reported that more than 80% of microplastics, which are stated as a structured feature in the sea water of the Black Sea, are composed of fibers and fragments (Terzi et al. 2022). This result supports the fact that the fiber was high in our study.

On the other hand, the densest length group of 500-1000  $\mu\text{m}$  (33%) was found in the MPs examined, followed by the length group of 118-500  $\mu\text{m}$  (30%). These size groupings are also visible in Figure 5 as belonging to PE and PP. The results obtained in the Black Sea by Eryaşar et al. (2022) are similar to the findings obtained. Apart from the study above, MP lengths range from 1001-2000  $\mu\text{m}$  in the Karasu River (Atamanalp et al., 2022), 1-2.5 mm in Hatay (Kılıç, 2022), <0.1 mm to 5.0 mm in Lake Van (Atici et al., 2022), and 1-3 mm in the North Pacific Ocean (Boerger et al., 2010) has been reported. The study's region of examination, the period during which samples were collected, and the various sampling and analytic techniques employed in the study all contributed to the inconsistencies between the studies.

Due to the fact that fish consume or ingest MPs, they can thus enter the body in both large and small particles (Atamanalp et al., 2022). In fish, MPs can cause digestive system disorders when they enter the body, causing problems that can negatively affect nutrition, growth, reproduction, and even survival (Foley et al., 2018). Although the toxic effects of MPs in fish and humans are not yet known, humans may experience effects such as oxidative stress, cytotoxicity, neurotoxicity, immune system disruption, and interstitial MP transfer after exposure to MPs (Bhuyan, 2022).

## CONCLUSION

This study focused on the presence and characterization of MPs in the GITs of horse mackerel caught off the Black Sea coast of Türkiye. With the data we gathered, we speculate that shifts in MP abundance depending on stations would be significant for fish habitats. However, further study is needed to link the biases in abundance, form, and quantity of MPs found in the marine ecosystem, particularly in semi-enclosed areas like the Black Sea. Future research should look at more species to pinpoint those most susceptible to MP contamination.

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