



Microplastic Occurrence in the Gastrointestinal Tract of Gray Mullet (*Mugil cephalus*) from Lake Cernek, Samsun, Türkiye

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Abstract: Microplastic (MP) ingestion by fish can not only impact the environment but also human health. This study determines the abundance and composition (polymer, shape, size) of MPs ingested by gray mullet (*Mugil cephalus*). A total of 30 specimens were purchased from local fish market collected from Lake Cernek located in Kızılırmak Delta, Türkiye by local fishermen. The digestive tracts of the fish were removed and digested with hydrogen peroxide (H₂O₂) to extract microplastics. The share of the contaminated fish with microplastics was 90%. The average microplastic abundance by individual and weight were 2.87±0.27 MP/ind and 0.014±0.001 MP/g respectively. The length, weight, and condition factor of the fish did not correlate with MP abundance (p > 0.05). Four different polymer types were determined in the digestive tract of the specimens. The composition of polymers consisted of 44.58% polyethylene terephthalate (PET), 34.94% polypropylene (PP), 10.84% polyethylene (PE), and 9.64% polyamide (PA). The determined shapes were fiber (67.47%), fragment (20.48%), and film (12.05%). The size range of MP particles was ranged between 132 µm to 4850 µm, and the average was 1522.45±131.22 µm.

Keywords: Fish, kızılırmak delta, microplastic, pollution, the black sea.

Cernek Gölü'ndeki (Samsun, Türkiye) Has Kefal (*Mugil cephalus*)'in sindirim sistemindeki mikroplastiklerin tespiti

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Öz: Balıkların mikroplastik tüketmesi sadece çevreyi değil aynı zamanda insan sağlığını da olumsuz etkileyebilmektedir. Bu çalışmada has kefal balığının (*Mugil cephalus*) tükettiği mikroplastiklerin miktarı ve kompozisyonu (polimer, şekil ve büyüklük) belirlenmiştir. Kızılırmak Deltası'ndaki Cernek Gölü'nden balıkçılar tarafından yakalanan 30 adet birey satın alınmıştır. Balıkların sindirim kanalı çıkartılmış ve mikroplastik ekstraksiyonu için hidrojen peroksit (H₂O₂) yardımı ile sindirilmiştir. Mikroplastiklerle kontamine olmuş balıkların oranı %90 olarak belirlenmiştir. Birey başına ve ağırlık başına mikroplastik bolluğu sırasıyla 2.87±0.27 MP/birey ve 0.014±0.001 MP/g'dır. Balıklarının boy, ağırlık ve kondisyon faktörleri ile mikroplastik bolluğu arasında anlamlı bir korelasyon bulunamamıştır (p > 0.05). Örneklerin sindirim sistemlerinde dört farklı polimer tipi belirlenmiştir. Bu polimerler %44,5 polietilen tereftalat (PET), %34,94 polipropilen (PP), %10,84 polietilen (PE) ve %9,64 poliamidden (PA) oluşmuştur. Belirlenen şekiller ise %67,47 fiber, %20,48 kırıntı ve %12,05 filmidir. Mikroplastik partiküllerinin büyüklükleri 132 µm - 4850 µm arasında değişmekte olup ortalaması 1522,45±131,22 µm olarak belirlenmiştir.

Anahtar kelimeler: Balık, karadeniz, kızılırmak deltası, kirlilik, mikroplastik.

INTRODUCTION

Plastic had become one of the main materials used in many industries. Since its first development, production has rapidly increased. In 2021, the global annual production reached 390.7 million tons (PlasticsEurope, 2021). This rapid increase in production and wide usage area has resulted in a large amount of plastic waste over time. Because of insufficient management, disposal, removal, and storage of plastic waste, several ecosystems were affected by plastic pollution (Liu et al., 2023; Tessnow-von Wysocki & Le Billon, 2019; Windsor et al., 2019). Among the plastic pollutants the ones less than 5 mm in length were named microplastics. These particles are mainly classified as primary and secondary microplastics (Cole et al., 2011). Primary microplastics are manufactured and intentionally used in consumer products, such as personal care products, cosmetics, and cleaning products. On the other hand, secondary microplastics result from the breakdown of larger plastic items, such as bags, bottles, and packaging materials, due to natural weathering or mechanical action.

A considerable amount of literature has been published on the composition, abundance, effects, and fate of microplastics in aquatic environments and organisms (Du et al., 2021; Tang et al., 2021). Accordingly, a global dispersal and a wide array of impacts on the aquatic environment and aquatic organisms were determined (Hamid et al., 2018; Jambeck et al., 2015). Ingestion of microplastics by aquatic organisms is known to be the most common pathway to the food chain (Carbery et al., 2018). Microplastics are small enough to be ingested by a wide range of aquatic organisms, including plankton, mollusks, crustaceans, and fish. Once ingested, they can cause a wide array of impacts on fish and also can be transferred through food web. For instance, exposure to microplastics has been linked to physical damage, altered gene expression, and changes in metabolism and immune function in fish (Mazurais et al., 2020; Sussarellu et al., 2019; Wu et al., 2020). Furthermore, microplastics can act as vectors for persistent organic pollutants and other toxic substances, which can accumulate in fish tissues and cause adverse health effects (Li et al., 2021; Zhang et al., 2020). Since there are multiple matrices such as abundance, polymer type, shape, size, and color, it is important to know the composition in nature to understand the adverse effects of microplastics.

Lake Cernek is located at Kızılırmak Delta, Samsun, Türkiye. It is one of the most important lakes for local fishermen. The lake receives pollutants from agricultural and domestic sources (Can & Taş, 2012). Microplastic pollution in different matrices including

seawater (Eryaşar et al., 2021; Terzi et al., 2022), and sediment (Akkan et al., 2023; Cincinelli et al., 2021; Terzi et al., 2022) were reported from the Black Sea. Previous studies in the Black Sea focused on economically important fish and mollusca species (Eryaşar et al., 2022; Gedik & Eryaşar, 2020; Gedik & Gozler, 2022). However, there is a data gap on MP pollution in freshwater ecosystems of north of Türkiye. Yet, there is no study reporting the MP occurrence and composition in flathead gray mullet (*Mugil cephalus*) in the Lake Cernek. Gray mullet is a cosmopolitan species and widely distributed in tropical, subtropical and temperate zones and has high salinity tolerance and can inhabit in marine, brackish and freshwater ecosystems (Crosetti & Blaber, 2015). Due to its wide distribution, it has been considered as a candidate bioindicator for microplastic pollution (Kilic & Yucel 2022; Reboa et al. 2022). Studies reported highly variable MP ingestion across its distribution range (Cheung et al., 2018; Guilhermino et al., 2021; Hastuti et al., 2019; Naidoo et al., 2016; Saha et al., 2021). In this context, this study determines the abundance and polymer, shape, and size composition of microplastics ingested by gray mullet (*Mugil cephalus*). The result of the study provides a comprehensive understanding to help research and prevention measures in the future.

MATERIAL AND METHOD

Sampling: A total of 30 gray mullets were purchased from a fish market in April 2021. The fish were caught by local fishermen from Lake Cernek, located in the Kızılırmak Delta of Türkiye (Figure 1). The standard length and weight of fish were measured, and Fulton's condition factor (Fulton, 1904) was calculated using the formulae: $K = W/L^3 \times 100$. Where K is Fulton's condition factor, W is the weight of the fish (g), and L is the standard length of the fish (cm).

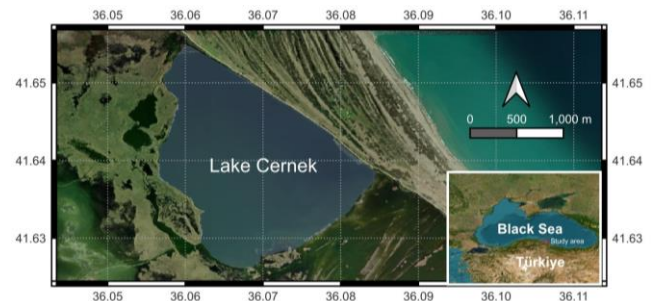


Figure 1. Study area and the location of Lake Cernek

Microplastic Extraction: The techniques used to extract MPs were carried out following the protocols outlined in Avio et al. (2015) and Jabeen et al. (2017). Each fish was rinsed with filtered pure water before dissection.

Each GIT removed was put in separate beakers. For the digestion process, %30 H₂O₂ solution (1:10 GIT: H₂O₂) was added to each beaker and exposed to 65°C for 5 days. Since there was sediment in the GIT of fish. Zinc chloride (ZnCl₂) was added to beakers and mixed with a glass rod to separate the MP particles from the sediment. Then the supernatant was filtered using Whatmann Grade 1 filter paper and kept in glass petri dishes until examination.

Examination and validation of the particles: The filters were eye-examined under a microscope. All the suspected MP particles on the filters were collected and moved to a clean filter for further examination. Suspected particles were photographed, and the properties of the particles (size, and shape) were determined based on the classification proposed by Hidalgo-Ruz et al. (2012). For MP validation and polymer determination Fourier Transform–Infrared Spectrometer (PerkinElmer Spektrum 100, ATR-FTIR) was used. The readings from FTIR were compared with the reference and the particles showing >70% similarity to the reference validated as microplastic.

Quality control: To prevent airborne and equipment-sourced microplastic contamination the following steps were followed. The extraction process was conducted in a private and controlled laboratory. No plastic equipment was used in the lab process. All equipment for dissection was rinsed with filtered pure water before each fish. All glass equipment (beakers, petri dishes, etc.) was cleaned with filtered pure water. The solutions and pure water used during the extraction process were filtered with GFC filter paper initially. To examine airborne contamination a blank beaker filled with filtered pure water was used as a negative control.

Data analysis: The data were expressed as mean±standard error of the mean. Spearman's correlation was used to determine the relationship between the size of the fish and MP abundance. The confidence interval was determined as 95%. Data analyses were conducted using R (ver. 4.2.1).

RESULTS

The average length, weight, and condition factor of the captured specimens were 31.06±1.51 cm, 472±15.9 g, and 1.16±0.01 respectively. Out of 30 specimens examined, 27 (90%) of them were found to be contaminated by microplastics. The average MP abundance by individual varied between 0 to 6 MP/ind with an average of 2.87±0.27 MP/ind. On the other hand, MP abundance by weight ranged between 0 to 0.03 MP/g and the average was 0.014±0.001 MP/g. The length ($r_s = 0.27$, $p > 0.05$) and weight ($r_s = 0.31$, $p > 0.05$) and condition factor ($r_s = 0.15$, $p > 0.05$) did not correlate with MP abundance.

The detected polymers from low to high were PET (polyethylene terephthalate), PP (polypropylene), PE (polyethylene), and PA (polyamide) (Figure 2A). The shape distribution was dominated by fibers (67.47%) and followed by fragments and film (Figure 2B). PA was dominated by the film (87.5%) shaped particles. PE consisted of all three shapes which were from high to low: fragment (44.4%), fiber (33.3%), and film (22.2%). The particle composition of PET is highly dominated by fiber (91.90%) and followed by fragment (5.41%), and film (2.70%). Two shapes were defined from PP which were fiber (62.10%) and fragment (37.90%) (Figure 2C). Fibers consisted of PET (60.70%), PP (32.10%), PE (5.36%), and PA (1.79%). The films were dominated by PA (70.00%) followed by PE (20.00%) and PET (10.00%). Fragments were PP (64.70%), PE (23.50%), and PET (11.80%) (Figure 2 D). The size of the MP particles varied between 132 µm to 4850 µm with an average of 1522.45±131.22 µm. Most of the particles were under 1000 µm (48.8%), 19.8% was between 1000-2000 µm and, 31.4% was > 2000 µm (Figure 2 E).

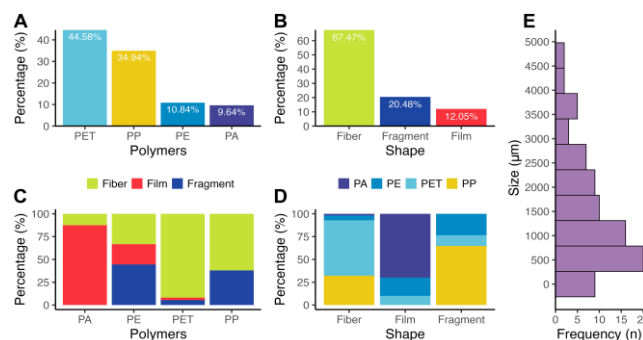


Figure 2. The distribution of polymers (a), shapes (b), polymers by shape (c), shapes by polymer (d), and size range of the polymers extracted from GIT of *Mugil cephalus*.

DISCUSSION

Mugilidae family is suggested as a candidate bioindicator for microplastic monitoring (Kilic & Yucel, 2022; Naidoo et al., 2016; Wootton et al., 2021; Zhang et al., 2020) in the aquatic environment. This study reports microplastic occurrence in gray mullet (*Mugil cephalus*) collected from Lake Cernek, Kızılırmak Delta, Türkiye. Gastrointestinal tract *M. cephalus* is contaminated with MPs which have different polymer types, sizes, and shapes. Almost all the specimens had MPs and the abundance was highly variable (0 to 6 MP/ind). No significant correlation between the size of the fish and MP abundance was determined. Similarly, Naidoo et al. (2016) and Zhang et al. (2020) reported no correlation between size and microplastic abundance for *M. cephalus*. There are numerous studies suggesting controversial results on the microplastic ingestion behavior of fishes. Different studies

have yielded different results regarding whether the consumption of MP by fish is a deliberate behavior. According to these studies, some fish species randomly ingest particles in their environment and prey during feeding (Li et al., 2021), while others consciously consume microplastic particles coated with biofilms because they resemble food such as fish eggs (Amaral-Zettler et al., 2015). Considering the variability and the lack of correlation between size and MP abundance it can be suggested that *M. cephalus* ingests MPs deliberately rather than a selective behavior.

The average number of MPs ingested by *M. cephalus* was 2.87 ± 0.27 MP/ind which was lower than most of the studied localities (Table 1). Previous studies on *M. cephalus* reported highly variable MP abundance ranging between 0.94 ± 0.18 MP/ind to 26.15 MP/ind (Table 1). The abundance and composition of ingested MPs are highly related to the environment (Bank, 2022). Furthermore, the extraction methods used can also affect

the results of the studies (Cole et al., 2014). The mentioned factors can be the cause for the high variability of MP abundance. Although the abundance was highly variable between the localities, the dominant shape of MPs extracted from the GIT of *M. cephalus* was fiber (Table 1). The results of this study agree with the dominant shape in the coastal waters (Terzi et al., 2022), some commercially important fish species (Eryaşar et al., 2022; Mutlu et al., 2022) and mollusks (Gedik & Eryaşar, 2020; Gedik & Gozler, 2022) from the Black Sea. Fiber shaped MPs in aquatic environments are mainly attributed to domestic washings of synthetic textiles (Browne et al., 2011). However, there are still data gaps for estimating the contribution of synthetic fibers to MP pollution in aquatic environments (Dris et al., 2016; Salvador Cesa et al., 2017). There is also evidence for high contribution of atmospheric fallout (Dris et al., 2015). The other potential sources of fibers in the study area are ropes used in fisheries activity and sacks used in agriculture practices.

Table 1. Studies examining variations in microplastic (MP) ingestion rates, abundance, and shapes of *M. cephalus* across different localities. (n.s. indicates not specified).

Locality	Percentage of Ingestion (%)	MP abundance (MP/ind)	Dominant shape	Reference
KwaZulu-Natal, South Africa	73	3.8 ± 0.53	Fiber	Naidoo et al. (2016)
China	100	3.7 ± 1.0	Fiber	Jabeen et al. (2017)
Sam Mun Tsai, Hong Kong	60	4.3	Fiber	Cheung et al. (2018)
Sydney Harbor, Australia	55	4.6 ± 7.8	Fiber	Halstead et al. (2018)
South Pacific subtropical gyre	13.6	2.0 ± 0.6	n.s.	Markic et al. (2018)
Jakarta, Indonesia	100	10.07 ± 6.4	Fiber	Hastuti et al. (2019)
Guangdong, South China	n.s.	5.23	Fiber	Zhang et al. (2020)
NE Atlantic Ocean	97	10 ± 9	Fiber	Guilhermino et al. (2021)
Southern Australia	50	0.94 ± 0.18	Fiber	Wootton et al. (2021)
Goa, India	n.s.	7.8 ± 4.4	Fiber	Saha et al. (2021)
East Anatolia, Türkiye	n.s.	6.71 ± 2.69	Fiber	Atamanalp et al. (2022)
Northern Mediterranean Sea	100	26.15	Fiber	Kılıç and Yücel, (2022)
Indonesia	n.s.	10.33	Fiber	Maulana et al. (2023)
<i>Kızılırmak Delta, Türkiye</i>	90	2.87 ± 0.27	Fiber	<i>This study</i>

The polymer composition was dominated by PET, and followed by PP, PE, and PA by order. Previous studies reported similar composition patterns in sea surface water (Eryaşar et al., 2021; Terzi et al., 2022), and mollusks (Gedik & Eryaşar, 2020; Gedik & Gozler, 2022) in the Black Sea. The mentioned polymers especially, PE, PET, and PP are the ones most produced by industry (PlasticsEurope, 2021). Thus, it is more likely to find those polymers in the environment. The study by Cheung et al. (2018) reports high abundance of polypropylene (42%) and polyethylene (25%) in *M. cephalus* GITs from Sam Mun Tsai, Hong Kong. Another study conducted on the same species from Sydney Harbor, Australia reported acrylic polyester (18%) and polyester (9%) dominated the polymer composition. It is worth mentioning that this study included non-synthetic and undefined particles to estimations.

The aquatic organisms are likely to ingest MP particles overlapping their prey size or smaller (Galloway et al., 2017). Thus, size is one of the most important factors effecting the bioavailability of microplastics. The share of the particles $<1000 \mu\text{m}$ was 48.8% in our samples. Similar

to polymer composition different results for *M. cephalus* were reported from different localities. Some reported results were $36.5\% < 2000 \mu\text{m}$ from China (Jabeen et al., 2017), $\sim 65\% < 500 \mu\text{m}$ from Guangdong, South China, and $\sim 45\text{-}60\% < 1000 \mu\text{m}$ from Türkiye. The size of ingested MPs also makes them available to be transferred through different trophic levels (Farrell & Nelson, 2013).

CONCLUSION

This study reports microplastic occurrence in GIT of *M. cephalus* collected from Lake Cernek. The abundance, size, shape and composition were variable. When compared to other studies on the same species from different localities, all properties of microplastics exhibited relatively different patterns except the shape. Thus, it is important to address the MP pollution in local scale since there are various factors effecting the MP composition such as socioeconomic structure, awareness level of local people, consumption habits, climate, water circulation and currents. More comprehensive research is needed to understand the effects of the MP particles found in the

aquatic ecosystems. The result of the study presents vital information for future research.

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