Turkish Journal of Zoology

The microplastic pattern in Turkish lakes: sediment and bivalve samples from Çıldır Lake, Almus Dam Lake, and Kartalkaya Dam Lake

KENAN GEDİK

ŞEBNEM ATASARAL

Recommended Citation

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Kenan GEDİK[1],* , Şebnem ATASARAL[2]

[1]Vocational School of Technical Sciences, Recep Tayyip Erdogan University, Rize, Turkey
[2]Faculty of Marine Science, Karadeniz Technical University, Trabzon, Turkey

Abstract: Plastic has become one of the most prominent contaminants in recent decades, posing a major environmental threat with critical implications for seafood safety. Thus, we investigated the abundance and characterization of the microplastics (MPs) in the sediment and bivalves from Çıldır Lake, Almus Dam Lake, and Kartalkaya Dam Lake in Turkey. The abundance of the MPs ranged from 19–156 MP kg^{-1} in the sediments, while 0.75–10.0 MP individual^{-1} (indiv.$^{-1}$) in *Anodonta sp.*, 0.16–1.00 MP indiv.$^{-1}$ in *Dreissena polymorpha*, 0.50–2.50 indiv.$^{-1}$ in the *Unio damescensis* was detected. ATR-FTIR was used to identify four distinct polymer types, with polyethylene terephthalate being the most prevalent. Fiber predominated in bivalve samples, whereas fragments in sediment and MPs were often <500 µm in length. Our data could serve as a foundation for a frequent monitoring routine in Turkish lakes since bivalves are one of the key vectors of MP contamination in humans.

Key words: Microplastic, mussel, freshwater, *Unio*, *Dreissena*, *Anodonta*

1. Introduction

Oceans gather a significant amount of litter from terrestrial sources, and >65 percent comprises nondegradable macroplastics (Thushari and Senevirathna, 2020). Relative abundance of microplastics (MPs) belonging to different particle size classes and polymer types are emerging ecological concerns in the 21st century. The reported ubiquity in various environmental compartments has increased lately (Peng et al., 2017: Berlund et al., 2019; Baldwin et al., 2020; Atici et al., 2021). This is mainly due to their persistence against natural degradation. However, due to UV exposure and mechanical weathering, large plastic fragments break down to form MPs, which are classified as a secondary source of MPs. In addition, another MP source is microbeads in personal care products (toothpaste, shampoos, etc.) classified as primary sources (Peng et al., 2017; Baldwin et al., 2020; Yang et al., 2021). These plastic particles accumulated in the cities are mainly shaped by socioeconomic class and customer behaviors across the globe (Siegfried et al., 2017).

As sessile, reasonably resistant to hazardous waste, and common in aquatic habitats across the world, the mussels are global biomarkers for their potential to detect the concentrations of chemical pollutants (Farrington et al., 2016). Although the freshwater bivalve studies in the

area are rapidly developing (Berglund et al., 2019; Baldwin et al., 2020; Hoellein et al., 2021), in vivo MP ingestion by bivalves in the urban littoral zones has received little attention up to date (Hoellein and Rochman, 2021). MP's origins, transport, and implications in freshwaters are still being discovered (Hoellein and Rochman, 2021). Few examples of Turkish inland water studies exist in the literature evaluating MP ingestion by fish (Atici et al., 2021) or MP concentration in fish species and the surrounding sediments (Turhan, 2022), frogs (Tatli et al., 2022) and freshwaters (Tavşanoğlu et al., 2020).

The employment of bioindicator species, which measure biological and biochemical characteristics over time, can be used to investigate contaminants in aquatic environments. In addition, the sessile species are beneficial for chemical contaminant monitoring (Farrington et al., 2016). Marine bivalves, for example, are one of the most reliable biological markers of aquatic contamination. However, there are inconsistent findings about the utility of bivalves as a reliable bioindicators for MP pollution (Su et al., 2018; Vescovi et al., 2009; Ward et al., 2019; Zhang et al., 2020; Hollein et al., 2022). The interaction between MPs and bivalves in Mediterranean mussels (*Mytilus galloprovincialis*) and Venus clam (*Chamelea gallina*) has been extensively studied in Turkish waters (Gedik and

* Correspondence: kenan.gedik@erdogan.edu.tr

Eryaşar, 2020; Gedik et al., 2022a; Gedik and Gözler, 2022), yet, there is only one research study on spatial or temporal patterns of MPs in bivalves inhabiting freshwater ecosystems of Turkey (Atici, 2022).

Plastics are derived and transported to the seas through freshwater habitats (Horton et al., 2017). However, there is no such program in Turkey as in the case of the US, for instance, that uses zebra and quagga mussels to monitor contaminants in the inland waters (Hoellein et al., 2021) regulated by North America's Great Lakes Mussel Watch Program. Nevertheless, understanding the dynamics of plastic contamination in Turkish lacustrine necessitates sorting the source and fate of MPs and their interactions with mussels. Therefore, our aims were: (i) to determine MP abundance, spatial distribution, and characterization in naturally growing bivalves and their surrounding sediments in the lakes and (ii) to provide a baseline for future monitoring studies.

2. Materials and methods

2.1. Characteristics of the study areas and sampled mussels

Located within the borders of Ardahan and Kars cities, Lake Çıldır has a surface area of 123 km^2 and is located at an elevation of 1959 m above sea level. The lake's surface is covered with a layer of ice during winter. While many little streams and rainfall that falls directly on the lake's surface provide the lake with water, there is only one stream to discharge the lake. Çıldır Lake, which was used as drinking water until recently, is now used for irrigation and energy production (Kükrer et al., 2014; Alkan et al., 2016).

Almus Dam Lake, located on the Yeşilırmak in Almus District of Tokat Province, was built for energy production, irrigation of agricultural areas, and flood control. In Almus Dam Lake, which has a surface area of 31.30 km^2, fish production facilities continue their activities (Buhan et al., 2010; Polat and Ozmen, 2011).

Kartalkaya Dam was built on the Aksu River to irrigate agricultural lands and supply drinking water. Although Kartalkaya Dam has a surface area of 10.25 km^2, there is a population of more than 100,000 in its basin in which intense agriculture and animal husbandry activities are carried out (Yücel et al., 2013; Özonat, 2017).

2.2. Mussel and sediment sampling

Mussel and sediments were sampled from Almus Dam Lake (ADL) and Kartalkaya Dam Lake (KDM) in October 2021 and Çıldır Lake (ÇL) in June 2021 by either free diving or scuba diving between depths of 0.5–3 m (Figure 1). Sediment sampling was carried out in triplicate. Sediment samples (approximately 2 kg) were taken from the sediment surface (top 5 cm) with a shovel. *Dreissena polymorpha* from ADL, *Unio damescensis* from KDL, and *Anodonta sp.* and D. polymorpha samples were collected

from ÇL. The number of bivalves sampled from the lakes is given in Table 1. Packed in an aluminum foil bowl and transported to the lab via a cold chain, the samples were stored at –20 °C in a refrigerator.

2.3. Microplastics extraction from mussel samples

After allowing the samples to thaw at room temperature (RT), they were rinsed with deionized (DI) water. Then, the length and weight of the mussels were measured (Table 1). Following, mussels' soft tissues were removed and placed in the glass flasks. Soft tissue pools were made using three separate samples of mussels with identical weights and lengths in each flask. For each station, five soft tissue pools were used (n = 5 replicates). In addition, at each station, two flasks with no tissue samples were utilized as blanks. All tissue samples were placed in aluminum foil-covered flasks with about 200 mL of H$_2$O$_2$ (30% Sigma Aldrich) and were incubated at 65 °C for 3 days manual shaking twice a day. After the incubation phase, the flasks were left out overnight (o/n) to enable them to reach RT. After vacuum filtering the samples, the flasks and glass funnel were rinsed with filtrated DI water to get materials off the side of the glass. To ensure a better visual inspection, multiple GF/C filters were used, depending on the quantity of the particles. For additional microscopic examination, each filter was kept in a separate, clean petri dish (Gedik and Eryaşar, 2020; Gedik et al., 2022ab; Gedik and Gözler, 2022).

2.4. Microplastic extraction from sediment samples

MPs were extracted from lake sediments using the density separation technique adopted from Hidalgo-Ruz et al. (2012). Sediments were dried for 48 h at 60 °C. The beakers were filled with a 500 mL volume of supersaturated ZnCl$_2$ (1.65 g cm^{-3}) after the transfer of 100 gr subsamples from each sediment sample. A glass stirring stick was used to mix the contents for 2 min. For around 4 h, the samples were allowed to settle down. A vacuum pump with a glass pipe was used to collect the supernatants, which were then filtered through a piece of plankton net used as a filter with a pore size of 25 μm. The plankton nets were rinsed with filtered DI water to get particles off the net and transferred into the new beakers. The whole procedure was performed three times using the initial sediment sample beaker to enhance the extraction of MPs from the samples. To digest the organic compounds in the new beaker containing particles extracted from the sediment samples, a 50 mL (30%) H$_2$O$_2$ solution was poured into the aluminum foil-sealed beakers to prevent air interference. The contents of the beakers were filtered using Whatman GF/C (47 mm diameter, 1.2 μm pore size filters) after digestion at 65 °C for 24 h. The filters were kept in glass petri dishes. In some cases, multiple filter papers were utilized to speed up microscopic observation due to the large number of particles in specific samples.
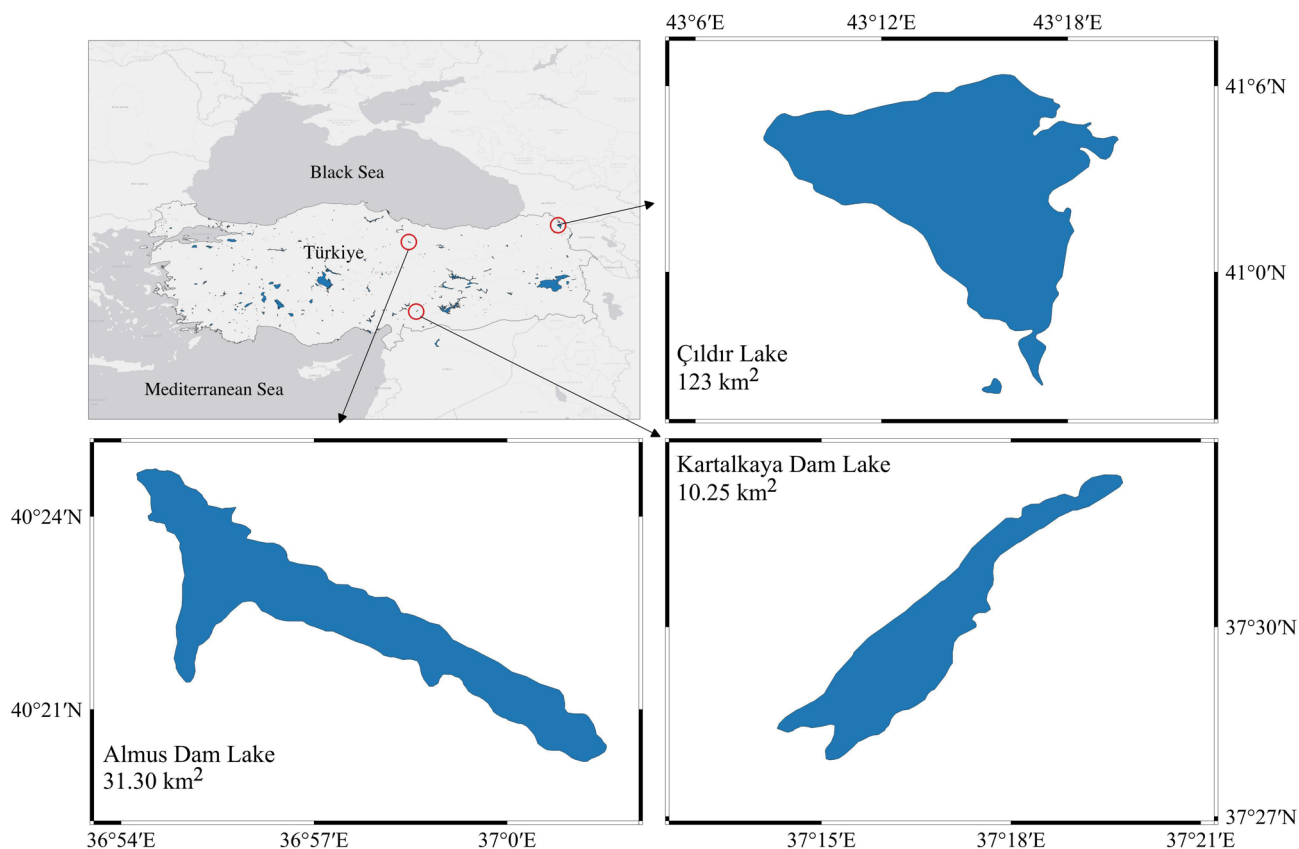
Figure 1. Sampling area. Red circles represent the locations of the lakes where sediments and mussels were collected.

Table 1. Morphometric characteristics of the bivalve sampled from different lakes.

Sampling location	Sampling species	N	L (cm)	BW (g)	FW (g)
Çıldır Lake	*Dreissena polymorpha*	36	2.64 ± 0.44	2.87 ± 1.14	0.96 ± 0.53
	Anodonta sp.	20	9.33 ± 1.20	43.60 ± 13.47	19.92 ± 7.39
Almus Dam Lake	*Dreissena polymorpha*	18	3.44 ± 0.05	2.05 ± 0.16	0.51 ± 0.01
Kartalkaya Dam Lake	*Unio damescensis*	16	7.92 ± 0.62	38.97 ± 6.72	12.97 ± 2.72

L: length, BW: total body weight, FW: soft tissue fresh weight. Results were given as average ± standard deviations.

2.5. Inspection and validation of the microplastic particles

Fluorescent staining is a faster and easier method for MP detection, rather than the combination of visual identification (Lusher et al., 2020) and the hot needle (De Witte et al., 2014) technique. One of the dyes used for this purpose is Nile red (NR) which several researchers have applied, and MPs have been detected successfully (Maes et al., 2017; Gedik et al., 2022b; Shruti et al., 2022). Therefore, NR was used in the filters in which the particles extracted from the sediment samples were collected in this study. NR (Sigma-Aldrich 72485) solution (1 mg mL^{-1}), the most used concentration in the literature (Shruti et al., 2022), was dissolved in the acetone, then filtered by a 0.22 μm PTFE stored in an amber bottle at +4 °C. A portion of the prepared solution was taken with a glass Pasteur pipette; a few drops were poured onto the filter to be examined (ensuring that the entire filter surface was wetted). An incubation period (approximately 30 min) was applied for the polymers to adsorb NR and the NR-stained filter papers to dry (Shruti et al., 2022). The filters were then inspected using a fluorescence microscope (Euromex oxion, filter set for blue excitation EX 465–495 nm DM 505 nm EM 515–558 nm, filter set for green excitation EX 540–580 nm DM

600 nm EM 605–665 nm). In biological samples, Nile red can also cause the MP overestimations calculated by dyeing undigested residues such as lipids and organic compounds that are not entirely broken down during digestion (Kang et al., 2020; Prata et al., 2021). For this reason, the filters obtained after the digestion of mussel samples were directly examined under the stereomicroscope. All the particles suspected to be MP were picked up by tweezers and placed into new filters. Using a digital camera, each particle from filters used for sediment and mussel samples was photographed, numbered, measured, and classed by fiber, foam, fragment, film, or pellet shape (Figure 2). Polymer identification was carried out with the help of an attenuated total reflection Fourier transform infrared spectrometer (PerkinElmer, FTIR) throughout a spectrum range of 4000–650 cm^{-1}, with 18 repeat scans (n) at resolutions of 4 cm^{-1}. The obtained data were compared to the library data from the Perkin Elmer instrument, and MPs were classified as particles that matched >70 percent of the cases (Figure 2) (Gedik and Eryaşar, 2020; Gedik et al., 2022ab). ATR-FTIR was used to evaluate 122 particles, of which 32 were separated from sediment samples, and 90 belonged to bivalve samples. Since the particles isolated from sediment samples were treated with Nile red, 32 particles among these were evaluated with FTIR by subsampling only the fluorescent particles, as opposed to all of the particles recovered from bivalve samples, which were all FTIR analyzed.

2.6. Contamination control and data quality

All processes were carried out in a clean and contamination-regulated setting. During the operations, linen gloves and lab coats were used. GFC filters (47 mm, 1.2 mm) were also used to prefilter all liquids used in the procedures. All assay glassware were cleaned with filtered DI before being covered with DI water-treated aluminum wrap. Washed petri dishes filled with filtered DI water were used as blank petri dishes and placed close to the instrument to check for contamination from the air during the visual check. The airborne contamination test detected only fiber particles from clothes in blank petri dishes. If there was a MP in these, the data was obtained by subtracting that from the total MP value of the series (Gedik et al., 2022ab).

The percent recovery efficiency of the MP extraction was measured using five different polymers with spiking methods by PE, PP, PA, PS, and PET particles to test the recovery. The gaining procedure of the spiking polymers was disclosed in Gedik et al. (2022). The MPs prepared for spiking were added to the blank glass flasks containing H$_2$O$_2$ (200 mL), which then received the same processes as those valid for the sediment samples. After filtration, the percent recoveries for PET, PE, PP, PS, and PA were 96%, 94%, 93%, 97%, and 92%, respectively.

2.7. Statistics

For mussels, data were expressed as MP individual (indiv.)$^{-1}$, MP fresh weight (fw)$^{-1}$, and MP kg^{-1} dry weight for sediment. Kruskal–Wallis and Mann–Whitney U tests
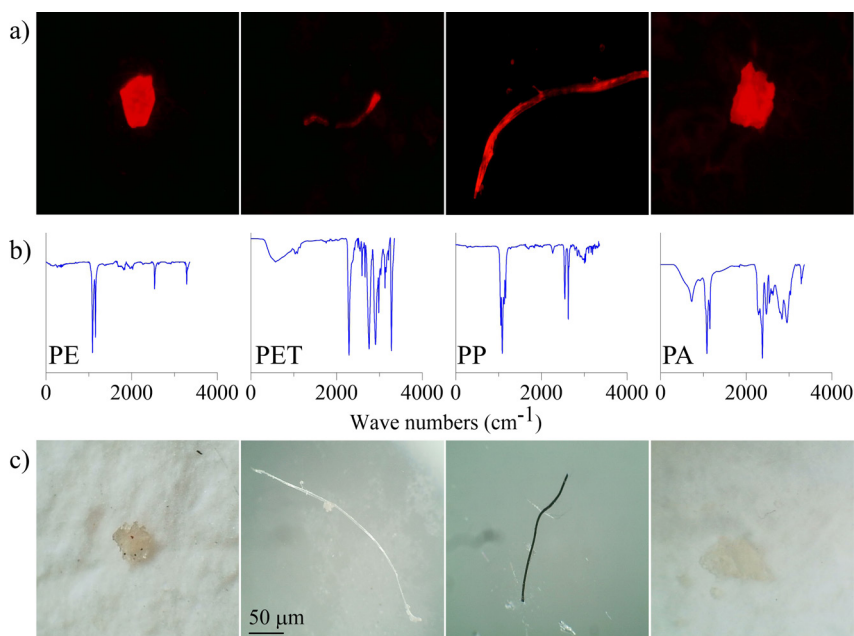

Figure 2. Evaluation of the extracted microplastics (MPs) from sediments and mussels. a) Appearance of MPs under a fluorescence microscope using Nile Red fluorescent dye, b) FTIR spectrums of MPs, and c) appearance of MPs under a stereo microscope.

were used to investigate any differences in MP abundance variability among different bivalves and sediments collected from different lakes. The significance interval was set at 0.05, and the software JMP 14.1.0 was used (SAS Institute Inc.).

3. Results

3.1. MP characterization
MPs were found in sediment and bivalve samples from all the lakes. ATR-FTIR analysis was performed on a total of 122 particles (Figure 2), of which 100 (82%) were MPs. The non-MPs were primarily composed of cellulose and other particles (which were not counted as MPs since they showed less than a 70% match).

3.1.1. Shapes
Three different types of MP (fiber, fragment, and film) were detected in the sediment and bivalve samples (Figure 3). While fragments were dominant (54%) in sediment samples, fibers (85%) were more prevalent in bivalves. Only fiber-type MPs were found in Almus Dam Lake sediments when the MPs obtained were classified according to their morphologies (Figure 4). While fragment (53.3%), followed by fiber (33.3%) and film (13.3%) in Lake Çıldır, the order of fragment (63.6%), followed by fiber (27.3%) and film (9.1%) was observed in Kartalkaya DL. When the shapes of MPs in bivalves were examined (Figure 5), 90% fiber, 8% fragment, and 2% film were found in *Anodonta sp.* sampled in the Lake Çıldır, while 100% of MPs were determined as fiber in *D. polymorpha.* There was 100% fiber in *D. polymorpha* sampled in Almus DL, while 45% of the MPs were found to be fiber, 45% to fragment, and 9% to film in the *U. damescensis* samples collected from Kartalkaya DL.

3.1.2. Polymer types
Four distinct polymer types were discovered in the sediment and bivalve samples (polyethylene, PE; polyamide, PA; polypropylene, PP; polyethylene terephthalate, PET) (Figure 3). PET was the dominant polymer type in sediment and bivalve samples. The polymer type amounts in the sediment and bivalves were as follows: PET (51%) > PP (29%) > PE (17%) > PA (3%) for sediments and PET (36%) > PP (32%) > PE (21%) > PA (11%) for bivalves (Figure 3).

According to their abundance in the sediment samples, the following polymer types were classified: PET (33.3%) > PE (26.7%) = PP (26.7%) > PA (13.3%) in Çıldır Lake, PET (50%) = PP (50%) in Almus DL, and PET (36.4%) = PP (36.4%) > PE (18.1%) > PA (9.1%) in Kartalkaya DL (Figure 4). Considering the MPs polymer types in bivalves (Figure 5), 50% PET, 35% PP, 13% PE, and 2% PA were detected for *Anodonta sp.* sampled from Çıldır Lake, while 60% PET, 20% PP, and 20% PE were determined for
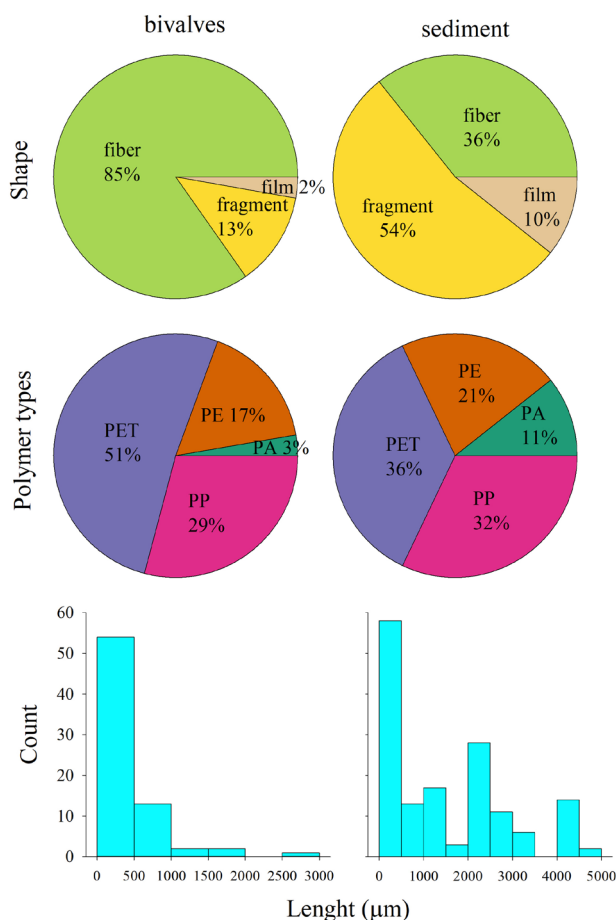

Figure 3. Characterization of microplastics (MPs) obtained from sediments and mussel samples. The upper panel is the shape, the middle is the polymer type, and the lower panel is the MPs' size.

D. polymorpha. A hundred percent PET was detected in *D. polymorpha,* collected in Almus DL. Conversely, 36% PET, 36% PE, 19% PP, and 8% PA were detected in *U. damescensis* (Figure 5).

3.1.3. Sizes
Figure 3 shows a histogram of the size variations of the detected MPs. The MPs found in the bivalve were on average 611 µm, ranging from 107 to 2967 µm. The majority of the MPs were found in the smallest class fraction (<500 µm), as indicated in the histogram in Figure 3. The mean of the MP length in sediment samples was estimated to be 1572 ± 1400 µm, with all MP lengths ranging from 57 to 4693 µm. <500 µm was the most typical size group (58%).

3.2. MP abundance in the sediments
MP distribution in sediment samples was 19 to 156 MP kg⁻¹. MP abundance in the sediment of the lakes was as follows: Kartalkaya Dam Lake (122 MPs kg⁻¹) > Çıldır
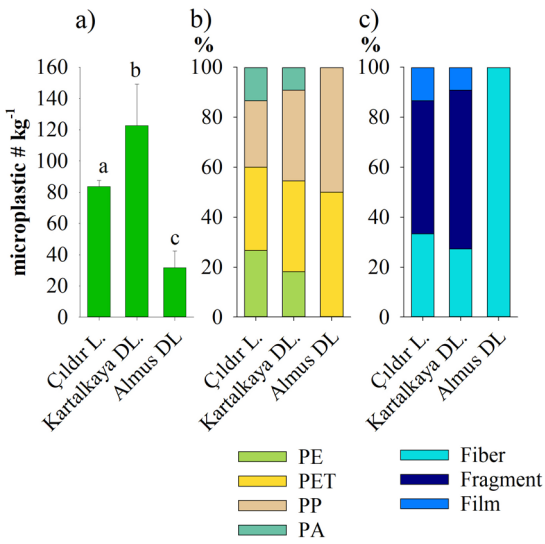
Figure 4. Abundance (a), polymer characterization (b), and shape (c) of microplastics collected from sediment samples from three lakes.

Lake (83 MPs kg^{-1}) > Almus Dam Lake (31 MP kg^{-1}). Kruskal–Wallis test was used to assess the MP abundance among sediments sampled from different lakes, revealing significant differences between the lakes (p < 0.05; Figure 4).

3.3. MP abundance in bivalves

The MP distribution measured for bivalves in the lakes ranged from 0.17 to 10.00 MP indiv.$^{-1}$ and 0.03 to 0.77 MP g^{-1} fw. When MP distribution in bivalves sampled from different lakes is examined (Figure 5), *Anodonta sp.* sampled in Lake Çıldır was 0.75–10 MP indiv.$^{-1}$, 0.03–0.41 MP g^{-1} fw; and *D. polymorpha* was 0.17–1.00 MP indiv.$^{-1}$, 0.12–0.77 MP g^{-1} fw. *D. polymorpha* sampled in Almus DL was 0.16–0.17 MP indiv.$^{-1}$, 0.32–0.33 MP g^{-1} fw, while 0.50–2.50 indiv.$^{-1}$ and 0.03–0.19 MP g^{-1} fw were detected in the *U. damescensis* sampled from Kartalkaya DL. The MP abundance in bivalves from different lakes showed statistically significant differences (Kruskal–Wallis test, p < 0.05).

4. Discussion

The aquatic ecosystem is under the pressure of various anthropogenic pollutants caused by industrial, urban, and agricultural activities (Vescovi et al., 2009; Hamza-Chaffai, 2014; Premalatha et al., 2020). Bioindicators that play a crucial role in assessing pollution levels and ecological hazards of contaminants are markers of this pressure in aquatic ecosystems (Su et al., 2018). Bivalves are useful sessile species that display the amounts of various contaminants in the environment among invertebrates (Boening, 1999; Su et al., 2018). In freshwater ecosystems,
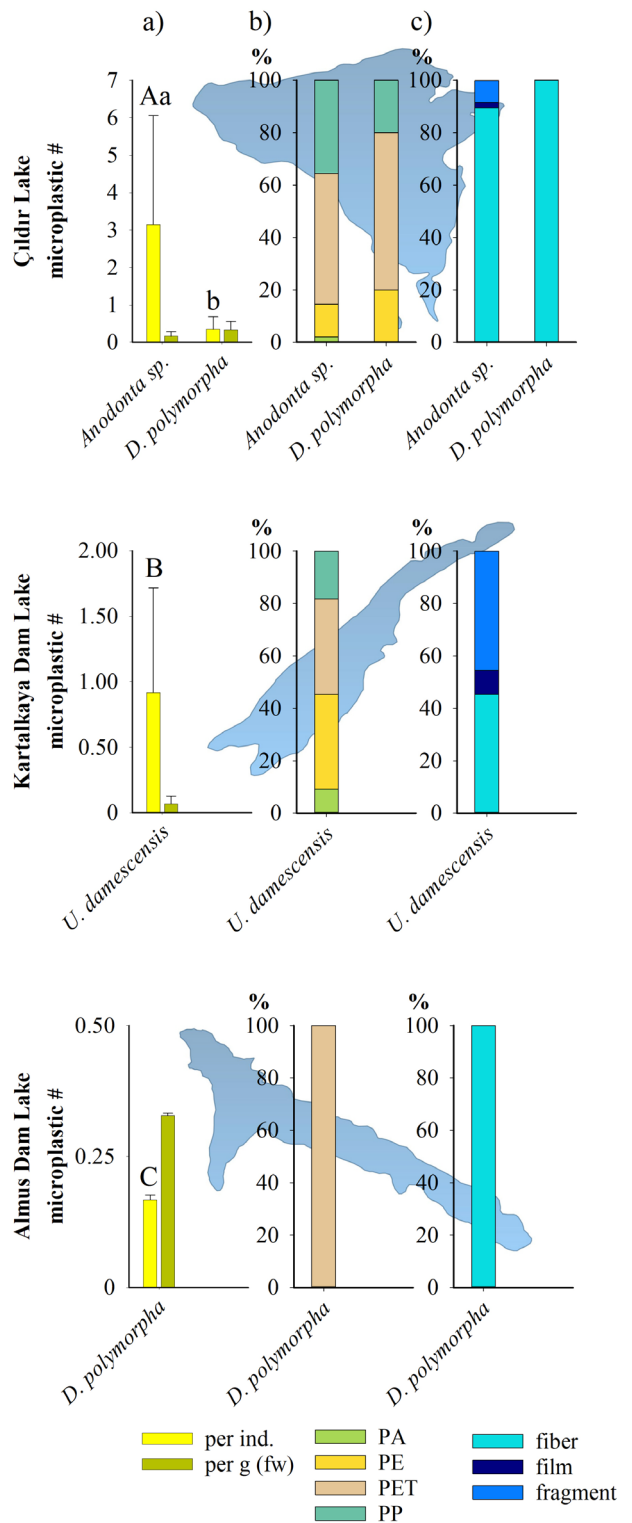

Figure 5. Microplastic abundance (per individual and g soft tissue fresh / wet weight) (a), polymer characterization (b), and shape (c) of microplastics collected from bivalve samples from three lakes. Different capitals (A, B, C) show significant differences among sampling stations. Different letters (a and b) show significant differences among bivalve species.

the necessity of examining the interactions of bivalves with microplastics, which exhibit major effects on ecosystem processes (Vaughn and Hoellein, 2018), such as water cycle and nutrient availability, is emphasized in the literature (Wardlaw and Prosser, 2020; Hoellein and Rochman, 2021; Hoellein et al., 2021). Therefore, monitoring MP contamination in our freshwater ecosystems might be essential. Thus, the MP abundance and characterization in sediments and bivalve (*Anodonta sp., D. polymorpha, and U. damescensis*) samples collected from Almus Dam Lake, Kartalkaya Dam Lake, and Çıldır Lake in Turkey were investigated in this work.

4.1. MP characterization

No municipal wastewater is discharged into the lake basin of Kartalkaya Dam Lake since drinking water is provided there (KBHKP, 2019). However, according to AÇDR (2020) and TÇDR (2020), there is no treatment for municipal discharge waters in Çıldır Lake and Almus Dam Lake. Direct or surface runoff is the two ways that pollutants enter the lake. According to Vardar et al. (2021), MPs predominated as fibers in municipal discharge waters. The intense presence of fiber in bivalves (Figure 5) may indicate that the pollution originates from municipal wastewater. Accordingly, Browne et al. (2011) reported that at least 1900 synthetic fiber particles are discharged together with wastewater in each washing machine use. In addition, it is thought that another source may come from synthetic equipment such as nets used in fishing (Andrady, 2011; Peng et al., 2017), because fishing or aquacultural production activities have been conducted in the lakes of Almus and Çıldır where the sampling was made (Buhan et al., 2010; Zengin et al., 2012).

The MP sizes detected in sediments and bivalves were predominantly <500 μm (Figure 3). Therefore, municipal discharge waters can be thought as a probable source of MP in mussel and sediment samples. De Falko et al. (2018) also reported that the length of the microfibers released with the washing of synthetic textiles is <500 μm. In the same study, De Falko et al. (2018) determined that more than 6 million microfibers were mixed into the wastewater due to washing 5 kg of synthetic fabric.

MPs detected in all samples were predominantly PET (36%–51%), PP (29%–32%), and PE (17%–21%) (Figure 3). The most produced polymer types in Europe are PE and PP, which are used to manufacture many plastic materials we use daily (Plastic Europe, 2020). Although PET, used extensively in beverage bottles and synthetic clothing fabrics, is produced less (Plastic Europe, 2020), it was more abundant in the sampling media due to municipal discharges. Accordingly, thousands of fiber MPs are released into wastewater while washing garments, according to Browne et al. (2011) and De Falko et al. (2018).

4.2. MP abundance in sediments

Microplastics accumulate substantially in the sediment, much like other contaminants (Belasi et al., 2020; Yang et al., 2021). The accumulation of MPs in the sediments of lakes can vary according to several factors such as the residence time of the water, surface area, MP density, MP composition, amount and number of municipal discharges, etc. (Corcoran et al., 2015; Eerkes-Medrano et al., 2015). Figure 4 depicts the number and distribution of MPs in the collected sediments from the research area. MP abundance in the sediments showed significant differences (Figure 4) among the lakes. These differences might be due to the urbanization, population density, and anthropogenic activities in the lake basin, as there is a link between these variables and MP contamination in lakes (Bellasi et al., 2020; Dusaucy et al., 2021; Yang et al., 2022). We suggest that MP density is high in Kartalkaya Dam Lake basin due to a higher population density than in other lakes and the intense livestock and agricultural activities (Yücel et al., 2013; Özonat, 2017). Çıldır Lake is also subject to anthropogenic activities, including fishing activities (Zengin et al., 2012) and municipal discharges given directly to the lake via rivers (Kükrer et al., 2014; Alkan et al., 2016). In terms of MP abundance, polymer type, and MP shape variety, the results show that Almus Dam Lake is more uniform than other lakes. Considering that MP abundance is related to anthropogenic activities, it can be concluded that Almus Dam Lake was less affected than other lakes.

To estimate the MP contamination levels of the sediments, the MP concentrations detected were compared with the research conducted in the other lakes in Turkey and around the world. Turhan (2022) performed a study in Sürgü Dam Lake (Turkey), and MP abundance was reported as 760–1440 MP m^{-2}. However, because our data were in MP kg^{-1}, a comparison with this study was impossible due to the unit discrepancy. The amount of MP detected in the sediments sampled from lakes by researchers worldwide is 0.7–7707 MP kg^{-1}, and the median value is 385 MP kg^{-1} (Dusaucy et al., 2021). The median value (83 MP kg^{-1}) we determined in our study is lower than the median value defined worldwide by various researchers in studies carried out on different continents. For instance, the median value in Lake Ulansuhai in China was 14–24 MP kg^{-1} (Qin et al., 2020), 0.77–0.92 MP kg^{-1} in Lake Tisza-tó, Hungary (Bordós et al., 2019), and 40 MP kg^{-1} in Lake Ziway, Ethiopia (Merga et al., 2020). On the contrary, studies conducted in rural and urban lakes of China detected 180–693 MP kg^{-1} (Yin et al., 2020), 250–300 MP kg^{-1} (Vaughan et al., 2017) in Edgbaston Pool (UK), 32.9–6229 MP kg^{-1} (Lenaker et al., 2019) in Lake Michigan (USA) and 1079.3 MP kg^{-1} (Oni et al., 2020) in Lake Ox Bow (Nigeria) which have obtained higher values

than ours. The differences between regions may be due to the differences in contamination levels as well as MP analysis procedure differences such as sampling, extraction of microplastics, filter pore diameter, FTIR verification, and contamination.

4.3. MP abundance in bivalves
The bivalves have been frequently used for monitoring MP pollution in aquatic systems (Li et al., 2019). Generally, in addition to the studies carried out in the marine environment (Gedik and Eryaşar, 2020; Gedik and Gözler, 2020; Gedik et al., 2022), they have also been used in freshwater systems (Hoellein et al., 2021; Pastorino et al., 2021). However, in studies conducted in Turkish lakes, MP was generally detected in water (Çomaklı et al., 2020; Erdoğan, 2020; Tavsanoğlu et al., 2020) and fish sampled in Lake Van (Atıcı et al., 2021). Bivalves have only been the subject of one study undertaken by Atici (2022), who sampled *Unio stevenianus* from the Karasu River, which drains into Van Lake, and analyzed MPs. In this study, MP characterization was performed in three different types of bivalve samples sampled from three different lakes in Turkey. Bivalve MP abundances from several lakes revealed statistically significant variations (Kruskal–Wallis test, p < 0.05, Figure 5). As is well known, bivalves are filtration feeders, meaning they get their food by filtering the water (Li et al., 2019). These differences in MP concentrations detected in bivalves may be simply due to the MP concentration differences in the waters of the lakes where they were sampled. The study did not include any water sampling; however, MP assessments were conducted in sediment samples, which are the main reservoir of contaminants. MP variations in bivalves were also detected in sediment samples (Figure 4). Based on this, it can be said that the lakes have different amounts of MP contamination. However, the MP concentration in their environment is not the only factor affecting MP accumulation in bivalves. Bivalve size can also be counted among these factors. Sampled bivalves were ordered according to their size (Table 1), as *Anadonta sp.* > *U. damescensis* > *D. polymorpha*. This ranking aligns with the MP concentration array detected in bivalve species (Figure 5). In Çıldır Lake, where two distinct species of bivalves were sampled, we can deduce that size is a significant component. Although tested from the same environment, the MP detected in *Anodonta sp.* was significantly higher than the amount of MP detected in *D. polymorpha.* (Figure 5, Mann–Whitney U test, p < 0.05).

Bivalves have also been observed to ingest fibers more readily than MPs of other forms (spheres, fragments, etc.) (Ward et al., 2019). For these reasons, our study overlaps with the literature data, in which we detected more fibers in bivalves. Similarly, MPs detected in bivalves sampled from the lakes were predominantly fiber (Hoellein et al., 2021; Pastorino et al., 2021).

The fact that bigger mussels filter more water and gather more MP in their bodies explains the phenomenon (Gedik and Eryaşar, 2020). Different researchers have also examined the relationship between bivalve size and MP accumulation, and some found a significant relationship between size and MP accumulation (Bråte et al., 2018; Berglund et al., 2019), while others did not (Phuong et al. al., 2018; Scott et al., 2019).

In the literature, just a few studies looked at the MP distribution in bivalves sampled from freshwater systems. When the MP values obtained in the bivalves tested in our study were compared with the lake studies in the literature, values show similarity with the studies conducted in Northern Italy (Pastorino et al., 2021) and Taihu Lake (*Corbicula fluminea*), China (Su et al., 2016). Yet, in the Great Lakes, USA study, the MP abundance detected in *D. polymorpha* (Hoellein et al., 2021) was higher than the values obtained here. In the study conducted in the Karasu River (Turkey), the amount of MP detected in *Unio stevenianus* was reported to be 39.15 ± 16.95 per individual and 2.85 ± 1.27 per g fw⁻¹ (Atici, 2022). These values were higher than the values in our study.

4.4. Are mussels a good indicator of microplastic?
Bioindicators are species or groups of species that reflect the degree of abiotic and biotic contamination in the ecosystem, according to Hadkinson and Jackson (2005). Filtration-fed organisms have a significant capacity to absorb pollutants from their environment (Jara-Marini et al., 2013; Su et al., 2018). While bivalves are also good bioindicator species for monitoring MPs reported by several researchers (Vescovi et al., 2009; Su et al., 2018, Zhang et al., 2020), other studies suggest otherwise. Ward et al. (2019) exposed bivalves to microspheres and microfibers of various sizes in their experimental work. The study revealed that the ingest ratio reduced as the size of the microspheres rose and that ingesting microfibers of any size was possible. Furthermore, they claimed that bivalves were poor bioindicators. In another work, Hoellein et al. (2021) did not recommend that mussels can be used as a bioindicator for MP monitoring. The results of our study were corroborated with the reports by Ward et al. (2019) and Hollein et al. (2021), confirming that mussels are not good bioindicators as MPs detected in the bivalves were predominantly fiber (85%), while MPs in the sediment samples were fragments (Figure 3). Similarly, the ratios of polymer types also differed (Figures 3–5). While more than half of the particles were PET in bivalves, a more homogeneous distribution was found in the sediments (Figure 3). Additionally, there was a noticeable variation in MP sizes (Figure 3). While the majority of the MP lengths found in bivalves were below 500 µm, a more homogenous distribution was observed in the sediments (Figure 3). Organisms must intake the majority of the plastic particles

they are exposed to be used as bioindicators in monitoring MP pollution, according to Ward et al. (2019). Our study findings align with the literature indicating that bivalves are not good biodindicators for MP pollution (the ones which mainly were fiber and < 500 μm) in the environment. This makes it possible to claim that bivalves are a poor choice of bioindicator for MP monitoring.

4.5. Human health
Seafood is a food group widely preferred by people across the world. However, with the increasing population and pollution of water resources, seafood is also contaminated with various pollutants. One of these pollutants is MPs (GESAMP, 2019). Many researchers have studied MP ingestion by seafood species; however, the majority of these have been conducted on marine organisms, including fish and bivalves (Bråte et al., 2018; Phuong et al., 2018; Scott et al., 2019; Gedik and Eryaşar, 2020; Eryaşar et al., 2022). Since all the soft tissue of the mussels is consumed among these species, the pollutants in the tissues are directly transmitted to humans. Researchers have determined the abundance of MPs in the mussel tissue intensively and calculated how much MP can be reached in humans with consumption rates (Van Cauwenberghe and Janssen, 2014; Catarino et al., 2018). As mentioned earlier, these studies were generally conducted on marine species. Studies in our country have also mainly been carried out on commercial species collected from the sea (Gedik and Eryaşar, 2020; Gedik et al., 2022; Gedik and Gözler, 2022). According to these studies, a weekly serving of 225 g (EFSA 2016) contains 52 MPs from *Mediterranean mussel*, 166 MPs from *Camelia gallina*, and 252 MPs from *Mediterranean mussel*, respectively. Although 42 different types of bivalves are detected today in our inland waters (Gürlek et al., 2019; Lopes-Lima et al., 2021), there is no data based on production, trade, or consumption, and MP contamination. Bivalves, *Anodonta sp.*, and *Unio damescensis* in particular (size and fresh weight, Table 1) found in inland waters might now serve as a substitute food source in the struggle against food crisis. If one serving (225 g EFSA, 2016) of the specified species *Anodonta sp.*

and *Unio damescensis* is consumed each week, one may be exposed to 38 and 15 MPs, respectively. These estimated levels are significantly less than the MP exposure that would result from consuming the aforementioned marine species. Mohammed Nor et al. (2021) calculated that the median value of MP taken by adults from 9 different media (tap water, air, mollusk, salt, milk, etc.) daily was 833. Another study calculated that individuals in the USA received an average of approximately 260 MPs per day (Cox et al., 2019). The ratio of MP taken from bivalves to the overall quantity of MP taken daily was calculated to range between 0.4% and 2.3% when the values we found in our study were compared to the amount of MP taken daily. Since the tolerated daily intake (TDI) for plastics has not yet been established, a valid comparison to a limit value cannot be made. Since the TDI values of some of the additives (for example, bisphenol A: 4 μg/kg bw/day EFSA, 2016) have been declared to date, future work might look into the relationship between MP and additives in bivalves.

4. Conclusion
In conclusion, MPs in different shapes and polymer types were detected in the lakes' sediments and bivalves where the sampling was performed. In this study, based on the MP's shape, type, and size, it is reasonable to indicate municipal discharge waters as a probable source of MP in the lakes. Furthermore, the gradual increase in sea bivalve production may also increase our country's inland water production potential. Therefore, continual contamination monitoring of bivalves used as human food will be beneficial.

Contribution of authors
SA and KG conceived the study. SA performed the fieldwork and provided the samples. KG performed the experiments. SA and KG wrote the paper. The authors read and approved the final manuscript.

Conflicts of interest
The authors declare no conflict of interest in this study.

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