



## RESEARCH ARTICLE

# Spatial Distribution of Microplastic Contamination in the Invasive Red Sea Mussel *Brachidontes pharaonis* (Fischer P., 1870) Around the İskenderun Bay

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## ABSTRACT

This study is first study reporting the microplastic abundance in soft tissues of a bivalvae *Brachidontes pharaonis* collected from 4 stations of İskenderun Bay. A total of 245 *B. pharaonis* specimens were examined and results showed that among examined specimens, 95 of them contained microplastic in their soft tissues. When all the data combined, mean MP abundance was found as  $0.4 \pm 0.5$  MPs ind<sup>-1</sup> and  $0.3 \pm 0.4$  MPs g<sup>-1</sup> ww. Fibers were predominant type of MPs and accounted for 75% of total extracted MPs, followed by fragments (25%). Majority of MPs were less than 1 mm and black. Fourier transform infrared spectroscopy (FTIR) showed that the extracted MPs were polypropylene (PP), polyethylene (PE), and polyethylene terephthalate (PET). Identified polymer types indicate that aquatic biota impacted by the anthropogenic influences such as agriculture, farming, fishing, household, etc. Results obtained in this study contribute the knowledge related with the microplastic contamination levels in marine biota.



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## 1. Introduction

Microplastics (MPs) defined as the plastic particles less than 5 mm (Arthur et al., 2009) are growing global problem. Especially after the establishment of the Marine Strategy Framework Directive which promotes to achieve good ecological status in terms of microplastic density in marine environments (European Commission, 2010), studies dealing with MP distribution, MP density and MP ingestion in aquatic animals are increased considerably.

So far, presence of MPs have been reported from marine environment i.e., sea surface (Suaria et al., 2016; Güven et al., 2017; Gedik et al., 2022a), seabed (Cheang et al., 2018; Erkan et al., 2021), and surface sediments (Wang et al., 2017; Abidli et al., 2018, Aytan et al., 2020). Since MPs are ubiquitous in marine environments, their interaction with marine bioata becomes inevitable. Till date, microplastic ingestion have been reported from many aquatic animals such as zooplankton (Beer et al., 2018), bivalve (Ding et al., 2021; Yozukmaz, 2021),

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crustacean (Yücel, 2022; Yücel & Kılıç, 2022), fish (Kılıç & Yücel, 2022). Among them, bivalves are more vulnerable to MP pollution due to their “extensive filter-feeding activity” (J. Li et al., 2019). Mussels, a member of bivalve class, are exposing to MP contamination by adherence in addition to the ingestion (Kolandhasamy et al., 2018). Laboratory studies showed that MPs intake reduces attachment strength, alters haemolymph proteome (Green et al., 2019), and affects the health and fecundity of mussels (Woods et al., 2018).

Mussels have a key function in benthic-pelagic ecosystem (Strayer et al., 1999). While mussels filter water from the water column, they concentrate the nutrients and particles found in the water column and create biodeposits which are transfer normally unavailable nutrients to the benthic organisms (Harris et al., 2021). In this way, they transfer the MPs found in the sea surface to the sea bottom. Recent study showed that MP presence cause variations in the sinking and resuspension rates (Harris et al., 2021) which lead to variations in the “ecosystem services provided by mussels” (Woods et al., 2018). In addition, MPs transfer to the upper trophic levels is also possible via predation (Santana et al., 2017; Renzi et al., 2018).

Due to their significant ecological role, global distribution, easy collection, high tolerance to environmental pollutants, sessile nature (reviewed from J. Li et al., 2019), mussels are widely used as bioindicator of environmental contaminants. Suitability of mussels as bioindicators of MP pollution have been evaluated by many researchers. Some studies showed that mussels are excellent bioindicator of MP contamination (Li et al., 2016; Fossi et al., 2018; J. Li et al., 2019; Ding et al., 2021) and gives information regarding MPs contamination in the both sea surface and littoral zone. Yet, some others report that mussels should be used as global indicators (Ward et al., 2019; Hoellein et al., 2021) which indicates that species based studies needs to be carried out to be obtain a clear result.

The present study investigates the microplastic ingestion in invasive *Brachidontes pharaonis* together with investigating the type, color, size of extracting MPs in the soft tissues. Results obtained in this study contribute the knowledge related with the microplastic contamination levels in marine biota.

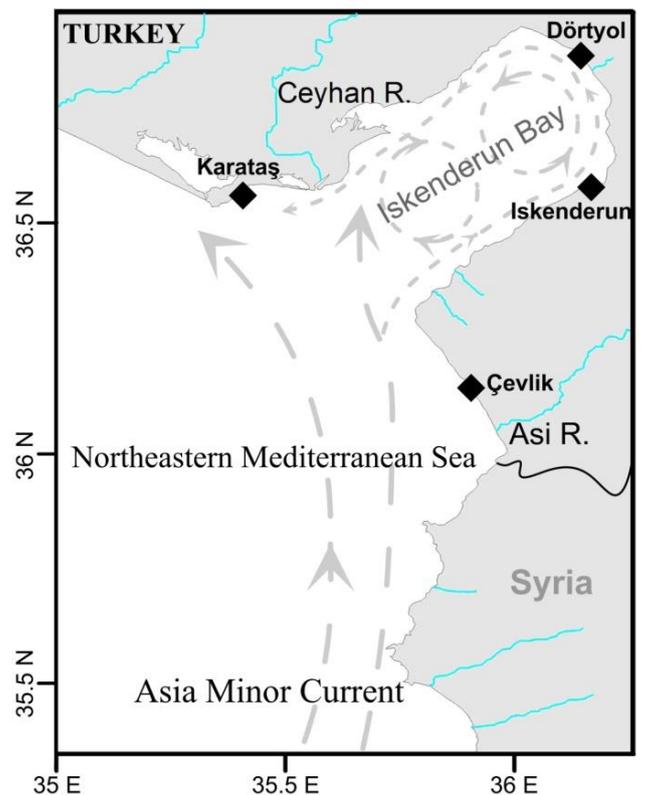
## 2. Materials and Methods

### 2.1. Study Area and Sampling

İskenderun Bay is located in the northeastern part of the Mediterranean Sea (Figure 1). Highly urbanized coastal area, intense industrial activities lead to discharge of a significant number of pollutants including MPs. Also, dredging activities applied in ports cause resuspension of MPs found in the sediment layer (Preston-Whyte et al., 2021), marine vessels and fishing equipment form an additional source of MPs (Nel et al., 2017). İskenderun Bay collects, accumulates and distributes this incoming pollution load depending on main surface flows

and dominant wind directions (Figure 1). Previous studies showed that microplastic pollution is significant in the İskenderun Bay (Güven et al., 2017) which result of microplastic presence in the gastrointestinal tract of marine fish (Kılıç, 2022; Kılıç & Yücel, 2022) and crustacea (Yücel, 2022).

In order to evaluate variations in the MPs accumulation levels along the bay, four monitoring stations (i.e., İskenderun, Çevlik, Dört Yol, and Karataş) were selected (Figure 1). From each station, 70-75 individuals of mussels which were close to the sea surface were randomly collected by hand with a metal knife in September 2022. The selected individuals were washed with distilled water and wrapped with tin foil. Then, they were placed in ice bag and transported to the laboratory.



**Figure 1.** Sampling locations (black rhombus) and sea surface currents [modified from Özsoy and Sözer (2006)] in the İskenderun Bay.

### 2.2. MP Extraction

At first, the surface of each specimen was cleaned with prefiltered pure water in order to prevent contamination. Then, each specimen was weighed and morphological characteristics were measured (i.e., shell length, width, and height). Next, the soft tissue of the mussel was removed by tweezer forceps carefully and weighed (wet weight). The soft tissue of each specimen was washed with prefiltered pure water and placed in separate glass beakers which were covered with tin foil. When the dissection process completed, 50 mL of H<sub>2</sub>O<sub>2</sub> was added to the beakers. Then, the beakers were placed on a hot plate and kept at 65 °C for 12 hours until the solution was homogenized

and soft parts were completely digested (Pazos et al., 2020). Finally, the remaining solution was filtered through 50 µm mesh filters and placed in a sterile glass petri dish.

### 2.3. Microscopic Examination and Polymer Identification

Filters were examined for the existence of microplastic particles by Olympus SZX7 microscope. Under microscope, each suspected particle was checked with hot needle to estimate the plastic nature. Identified particles were classified according to type (fiber, fragment), color (black, red, blue, white, transparent, green, brown, silver and yellow) and size. Filters which have MPs larger than 1 mm were placed in glass petri dishes and set aside for Fourier transform infrared (FTIR) analysis.

To evaluate the polymer configuration of detected particles, Fourier transform infrared spectroscopy (FTIR) was employed. At this stage, out of 104 MPs, 28 MPs suitable in size were used as subsamples and analyzed for polymer identification. FTIR analysis was carried out on a SHIMADZU QATR10 FTIR spectrophotometer equipped with single reflection attenuated total reflectance (ATR) accessory. The spectrum range was arranged as 4000-400 cm<sup>-1</sup> and a resolution was set to 4.0 cm<sup>-1</sup> with 32 scans for each measurement. The polymer type was identified by comparing absorbance spectra to reference libraries of SHIMADZU library

#### 2.3.1. Contamination prevention

The critical step of microplastic examination is contamination prevention. For that reason, comprehensive precautions were employed to prevent airborne contamination. First, each step of the study was performed in closed laboratories with restricted access. Only authorized personnel were allowed to enter the laboratories and they wore nitrile gloves and cotton aprons at all times. Before any analysis, laboratory surfaces, dissection equipment and glass beakers were cleaned twice with prefiltered distilled water. Filters and sterile petri dishes were checked under the microscope for the presence of MPs before use. Three wet blank filters were placed in the laboratory during dissection and microscopic examination steps. Even though we applied all necessary precautions, 1 fiber particle was detected in one blank filter (out of 6 blank filters). Particle detection rate in the blank filters was 0.17±0.41 which indicates that results were scientifically acceptable. The data was corrected by extracting the contamination data.

### 2.4. Statistical Analysis

Since normality of the dataset could not be verified by the Shapiro-Wilk test, PerMANOVA which is a non-parametric test of significant difference was employed to detect variations

in the MPs abundance between stations. Non-parametric spearman correlation analysis was applied to test correlation between morphological features of *Brachidontes pharaonis* (i.e., shell length, height, weight, wet weight) and MPs abundance. Significance level of 0.05 was set for all statistical computations (p<0.05).

## 3. Results

In this study, total of 245 mussels, collected from 4 different stations along to İskenderun Bay, were examined. Mean length and weight of all examined mussels were found as 15.3±13.7 mm and 3.0±0.9 g, respectively. Information regarding other morphological averages depending on stations were given in Table 1.

A total of 104 MPs particles were detected (Figure 2). When all the data combined, mean MP abundance was found as 0.4±0.5 MPs ind<sup>-1</sup> and 0.3±0.4 MPs g<sup>-1</sup> ww. Depending on stations mean MP abundance was varied from 0.2±0.5 MPs ind<sup>-1</sup> to 0.6±0.6 MPs ind<sup>-1</sup> and from 0.1±0.3 MPs g<sup>-1</sup> ww to 0.4±0.5 MPs g<sup>-1</sup> ww (Table 1). The highest MP quantity per individual and per soft tissue (ww) were detected in the Çevlik station. Whereas, the lowest MPs amount per individual and per soft tissue (ww) were found in İskenderun station. The microplastic abundance in the mussels collected from Çevlik stations were found to be significantly different than other stations in terms of both MPs per individual (ind) and MPs per soft tissue (ww) (p<0.05). In addition, MPs accumulation in terms of MPs per gr, MPs accumulation levels between Karataş and İskenderun, Dörtüol and Çevlik, Dörtüol and İskenderun showed significant variations (p<0.05).

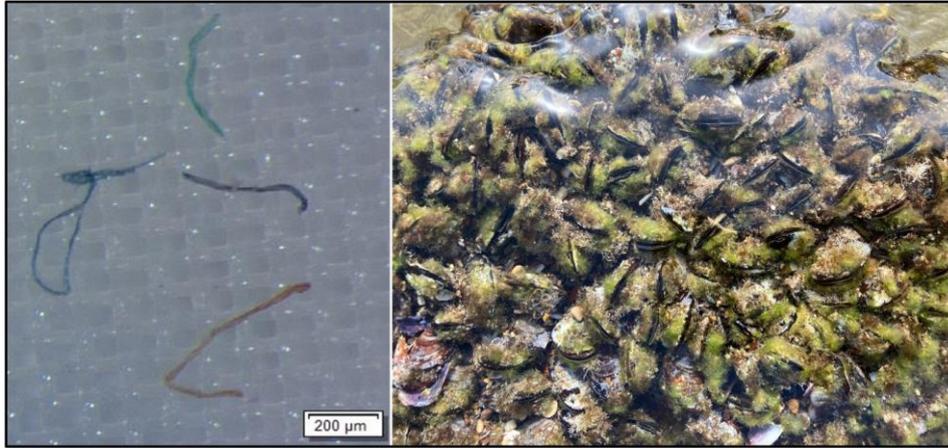
Detected MPs were divided into two categories as fiber and fragment. Fiber were the most commonly found microplastic type, accounting for 75% of total extracted MPs. Rest of the extracted MP particles (25%) were fragments (Figure 3). Pellet shape MPs which is major outcome of primary sources were not detected in this study.

In terms of color, approximately half of the extracted MP particle were black (52%) and followed by blue (31%), red (7%), green (7%), brown (3%), and silver (1%), respectively (Figure 3). Size of detected MPs were varied from 80 µm to 3150 µm with a mean of 875±675 µm. Majority of the extracted MPs were smaller than 1000 µm in size.

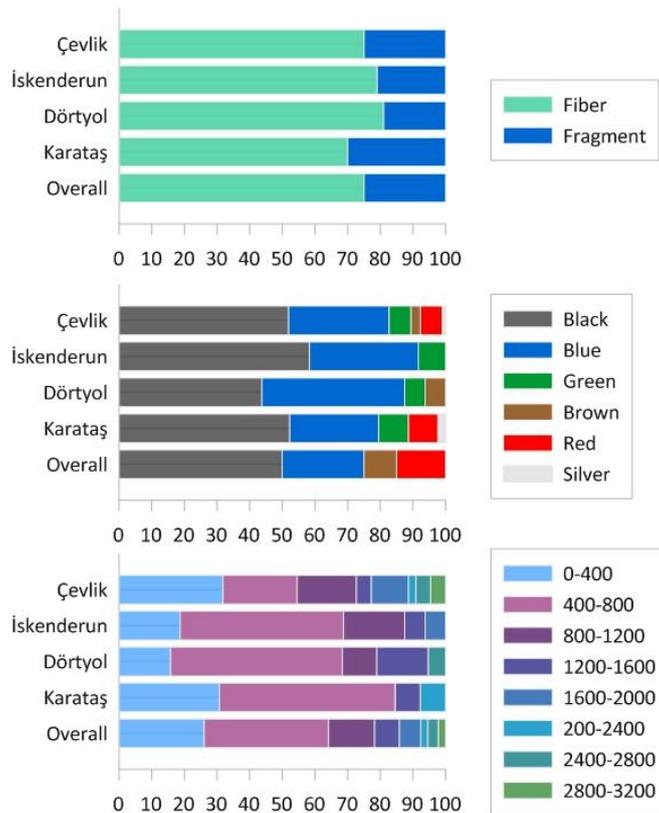
In this study, the origin of 27% of the total extracted MPs was used in the FTIR analysis. Among 28 examined MPs, 4 suspected particles were found to be organic in nature and polymer type of 5 suspected particles could not be determined. Remaining particles were found to be polyethylene (PE) [32%], polypropylene (PP) [21%], polyethylene terephthalate (PET) [14%].

**Table 1.** Microplastic abundance in the soft tissue of *Brachidontes pharaonis* along the İskenderun Bay, Türkiye.

Station	n	Length (mm)	Width (mm)	Height (mm)	Total weight (g)	Soft tissue weight (g)	Total number of MPs	MPs abundance (MPs ind <sup>-1</sup> )	MPs abundance (MPs gr <sup>-1</sup> ww)	Occurrence rate (%)
Karataş	70	25.8±10.1	11.4±4.4	10.6±4.4	2.7±1.0	1.4±0.5	24	0.3±0.5 <sup>a</sup>	0.3±0.5 <sup>ab</sup>	33
Dörtyol	70	14.9±7.6	5.1±3.1	8.1±3.1	2.9±0.8	1.5±0.4	20	0.3±0.5 <sup>a</sup>	0.2±0.3 <sup>ac</sup>	27
İskenderun	70	15.0±8.6	6.1±2.9	7.2±3.4	3.2±0.9	1.6±0.4	16	0.2±0.5 <sup>a</sup>	0.1±0.3 <sup>c</sup>	21
Çevlik	75	24.7±11.2	10.7±4.8	9.8±4.7	3.0±0.8	1.5±0.42	44	0.6±0.6 <sup>b</sup>	0.4±0.5 <sup>bc</sup>	50



**Figure 2.** Representative image of fibers extracted from the soft tissues of *Brachidontes pharaonis* (left), photograph of *Brachidontes pharaonis* colony (right).



**Figure 3.** Percentage distribution of MPs characteristics in terms of shape (above), color (middle), and size in µm (below).

#### 4. Discussion

İskenderun Bay is located inside the biggest plastic hotspot of the Mediterranean Sea (Papadimitriou & Allinson, 2022). As result of urbanized coastal line and intense industrial activities, severe microplastic pollution has been reported in the study area (Güven et al., 2017). Studies evaluating microplastic abundance in marine biota in the İskenderun Bay have been limited with fish (Kılıç, 2022; Kılıç & Yücel, 2022) and crustacea (Yücel, 2022) and missing in terms of bivalves. This study is first study reporting the microplastic abundance in soft tissues of a red sea mussel *Brachidontes pharaonis* from İskenderun Bay.

Mussels are sessile species and they can uptake microplastic particles from marine environments while filtering the water. In addition laboratory studies have demonstrated that MP uptake could be driven by adherence (Kolandhasamy et al., 2018) and fusion by mussel byssus (Q. Li et al., 2019). In this study, almost half of examined *Brachidontes pharaonis* specimens contained MPs in their soft tissues (45% of 245 specimens). Considering their biological mechanisms of mussels, results demonstrate that MP contamination is inevitable when MPs are present in the littoral zone. Similar to this study, previous studies also showed that mussels can uptake significant amount

of microplastics in their soft tissues (Pedersen et al., 2020; Wakkaf et al., 2020; Joyce & Falkenberg, 2023).

Since this study is the first attempt to report microplastic abundance in the *Brachidontes pharaonis*, results could only be compared with studies employing other mussel species. Previous studies reported higher MPs occurrence rates than the results of this study. The MPs occurrence frequency in the *Mytilus galloprovincialis* as 46.3% in northern Ionian Sea (Digka et al., 2018), 64% in Turkish coastline (Gedik & Eryaşar, 2020), 97% in Bizerte lagoon, northern Tunisia (Wakkaf et al., 2020). Joyce and Falkenberg (2023) reported the microplastic occurrence in the soft tissues of *Brachidontes variabilis*, *Perna viridis*, *Xenostrobus securis* as 67% from Hong Kong. Lastly, microplastic occurrence rate in the *Limnoperna fortune* from Río de la Plata estuary, Argentina was reported as 96% (Pazos et al., 2020).

Microplastic abundance in terms of both MPs per ind and MPs per gr (ww) were consisted with the previous reports (Table 2). Only exception is Digka et al. (2018) who reported higher MPs accumulation rates than this study. Different from this study, they used gills and digestive glands of the mussels rather than whole soft tissue of mussel which might cause variations in the MPs accumulation.

**Table 2.** Some studies reporting the microplastic abundance in mussel species.

Location	Species	MPs abundance (MPs ind <sup>-1</sup> )	MPs abundance (MPs gr <sup>-1</sup> ww)	Dominant type	Dominant polymer	References
İskenderun Bay, Türkiye	<i>Brachidontes pharaonis</i>	0.4±0.5	0.3±0.4	Fiber	PE, PP	This study
Aegean Sea, Türkiye	<i>Pinctada imbricata radiata</i>	2.16	-	Fiber	PE	Aksakal et al. (2021)
Bizerte lagoon, Northern Tunisia	<i>Mytilus galloprovincialis</i>	0.4±0.2	2.1±1.0	Fiber	PE, PP	Wakkaf et al. (2020)
Tolo Harbour, Hong Kong	<i>Brachidontes variabilis</i>	0.2-0.7	0.9-3.1	Fiber	-	Joyce and Falkenberg (2023)
Tolo Harbour, Hong Kong	<i>Perna viridis</i>	0.6-3.1	2.4-15.1	Fiber	-	Joyce and Falkenberg (2023)
Tolo Harbour, Hong Kong	<i>Xenostrobus securis</i>	2.88-14.67	1.5-8.4	Fiber	-	Joyce and Falkenberg (2023)
Marmara Sea, Türkiye	<i>Mytilus galloprovincialis</i>	0.30 -7.53	0.11 to 4.58	Fiber	PET	Gedik et al. (2022b)
Black Sea	<i>Chamelea gallina</i>	0.22–2.17	-	Fiber	PET, PE, PP	Gedik and Gozler (2022)
Pays de la Loire region, France	<i>Mytilus edulis</i>	0.60±0.56	0.23±0.20	Fragment	PE, PP	Phuong et al. (2018)
Turkish coast	<i>Mytilus galloprovincialis</i>	0.06-2.47	0.02-1.12	Fragment	PET, PE, PP	Gedik and Eryaşar (2020)
Río de la Plata estuary, Argentina	<i>Limnoperna fortune</i>	0.43±0.35	2.08±1.33	Fiber	-	Pazos et al. (2020)
Northern Ionian Sea	<i>Mytilus galloprovincialis</i>	1.9±0.2	5.3±0.5	Fragment	PE	Digka et al. (2018)

Even though MP uptake is common report in all mentioned studies, variations observed in MPs accumulation and occurrence rate are arise from many environmental and methodological differences. First of all, since these species sessile, their MPs ingestion is primary affected by the MPs concentration in the surrounding environment. In addition, environmental factors such as salinity (Khoironi et al., 2018) and season (Ding et al., 2021) cause variations in the microplastic uptake. Lastly, lack of common applied methodology makes comparison harder since different methodologies may lead to under or overestimation.

In this study, MP accumulation in terms of MPs per ind and MPs per gr were significantly higher in Çevlik station ( $p < 0.05$ ). Significant amount of plastic were transported to coastal part of Çevlik station due to main current system and wind directions (Yılmaz et al., 2022). In addition, many waste deposition peaks were formed around the discharge zone of Orontes River (Yılmaz et al., 2022) which seems to be increase the microplastic amount in the coastal shore. In a basic sense, the higher microplastic contamination in the habitat of *Brachidontes pharaonis*, the higher the microplastic accumulation in the mussel. This tendency has been also demonstrated by previous studies (Li et al., 2016; Phuong et al., 2018).

Statistical analysis did not reveal a correlation between mussel length and MP abundance as well as mussel weight and MP abundance. Similar outcome was also reported in previous reports in mussels (Phuong et al., 2018; Scott et al., 2019; Gedik et al., 2022b). This outcome clarifies that MPs accumulation is mostly depended on the contamination level in the animals' habitat rather than its size.

Shape of MPs is directly related with the bioavailability of MP particle since it affects their accumulation potential and adverse effects (Qu et al., 2018; Fernández & Albentosa, 2019). Only fiber and fragment MPs were detected in this study, which is reported to be most commonly extracted MPs shapes (J. Li et al., 2019). We did not find any pellet shape MPs which is probably related with the selective ingestion of *Brachidontes pharaonis*. Because, Alnajar et al. (2021) detected that filter feeding mussels do not ingest regular shape particles (i.e. pellet, microbead) under controlled conditions. On the other hand, fiber shape MPs more easily ingested by mussels and get trapped in the gills and hepatopancreas (Renzi et al., 2018). In parallel with this, majority of the extracted MPs (75%) were fiber in shape which coincide with previous studies in mussels (Li et al., 2016; Qu et al., 2018; Renzi et al., 2018; Pazos et al., 2020; Wakkaf et al., 2020; Ding et al., 2021; Yozukmaz, 2021; Gedik & Gozler, 2022; Gedik et al., 2022b; Joyce & Falkenberg, 2023). A recent study showed that microfibers act like a vectors leading to an indirect toxicity of chemicals, metals, monomers, dyes (Alnajar et al., 2021).

On the other hand, dominance of fragment shape MPs was also reported in many studies (Digka et al. 2018; Phuong et al. 2018; Gedik & Eryaşar, 2020). According to the Digka et al. (2018), variations in the predominant shape is related with the anthropogenic activities around the study area such as poor waste management strategies, recreational fishing and tourism. These activities are reported to be increase the fragment type MPs in the marine environment. Similarly, poor sewage system could cause increase in the fiber shape MPs since millions of fibers could be released from a single laundry (Galafassi et al., 2019).

Size of extracted MP was is usually limited with the size of the animal (Jâms et al., 2020). In line with this, small size MP (<1 mm) particles were dominant in this study. Similar observation was also reported in the previous studies (Li et al., 2016; Brâte et al., 2018; Digka et al., 2018; Pazos et al., 2020; Gedik et al., 2022b). While considering the lab scale studies which reporting the translocation of small size MPs in the circularity systems and other organs (Browne et al., 2008; von Moos et al., 2012), dominance of small size MPs might also be an outcome of translocation and accumulation besides ingestion organs.

In terms of color, black colored MPs were dominant in all examined stations which overlap with the previous results reporting the MPs abundance in the fish (Kılıç, 2022; Kılıç & Yücel, 2022) and crustacea (Yücel, 2022) samples from the İskenderun Bay.

Polymer type is an important parameter which influences the uptake of MPs by mussels. In this study, PE, PP, and PET were the dominant type of polymers which is similar with the previous findings (see Table 2). These polymers were globally most demanded and produced polymers (Plastic Europe, 2022) which increase the exposure risk of marine biota. They are widely used in many areas including but not limited to agriculture, farming, fishing ropes, household, water bottles, packaging (Plastic Europe, 2022).

## 5. Conclusion

Till date, most of the research was focused on commercially important mussel species, while neglecting other "ecosystem engineering species" (Joyce & Falkenberg, 2023). This study is first study reporting the microplastic abundance in soft tissues of a bivalve *Brachidontes pharaonis* from İskenderun Bay. Results showed the severity of MPs contamination in the region. Fibers were the major dominant type of polymers which demonstrates the pressure of highly urbanized and industrialized coastal area. Polypropylene and polyethylene are the main polymer types which also indicate the impact of anthropogenic activities in the marine biota. More studies needs to be carried out to understand the MPs transport in the study area.

## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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