Hydrobiologia
https://doi.org/10.1007/s10750-026-06271-3

AQUATIC PLASTIC POLLUTION

Pollution status of microplastics in freshwater basins of Türkiye: evidence from all *Barbus* (Daudin, 1805) species

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Received: 12 March 2026 / Revised: 30 May 2026 / Accepted: 6 June 2026

Abstract Microplastic (MP) pollution in freshwater ecosystems is a growing global concern, yet long-term data tracking its historical progression remains scarce. This study provides a retrospective analysis of MP ingestion in 12 *Barbus* species collected from five major hydrological basins in Türkiye between 2004 and 2018. A total of 373 collection specimens were examined to quantify MP abundance, characterize polymer types using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, and evaluate spatio-temporal patterns. Microplastics were detected in 19.8% of the individuals, with an average abundance of 0.27 MP/individual. The Caspian Sea basin exhibited the highest observed MP concentrations (1.67 MP/individual), though no statistically significant differences were determined among basins. Fibers were the dominant morphology, and ethylene–vinyl acetate and polyamide were the most frequent polymer types. Notably, MP ingestion was persistent throughout the study period, indicating a chronic presence of plastic in Turkish freshwater systems rather than a significant temporal trend. The results suggest that benthic *Barbus* species serve as suitable indicators for monitoring sediment-associated MP pollution, although their capacity to differentiate localized pollution gradients requires further investigation. This study underscores the role of museum collections in bridging historical data gaps

Guest editors: Massimiliano Scalici, Paula Sobral, Timothy Hoellein & Verónica Ferreira / Plastic Pollution in Aquatic Ecosystems

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Published online: 22 June 2026

and provides a baseline for future freshwater management strategies.

Keywords Freshwater · *Barbus* species · River · Spatiotemporal · Microplastic

Introduction

Plastic pollution has emerged as one of the most critical environmental challenges of the twenty-first century, increasingly recognized as a persistent stratigraphic marker of the Anthropocene (Zalasiewicz et al., 2016). Specifically, microplastics (MP), plastic particles smaller than 5 mm, pose a global threat due to their ubiquitous distribution and capacity to infiltrate diverse ecological niches (Eerkes-Medrano et al., 2018). While historical research has predominantly focused on marine environments, recent scientific attention has shifted toward freshwater ecosystems, such as rivers, lakes, and reservoirs. These inland waters serve not only as primary conduits for transporting terrestrial plastic debris to the oceans but also as significant sinks for MP accumulation due to intense anthropogenic pressures (Wagner et al., 2014; Horton et al., 2017).

The sources of microplastics in freshwater systems are multifaceted, encompassing municipal wastewater effluents, industrial discharges, agricultural runoff, and atmospheric deposition (Siegfried et al., 2017). Monitoring pollution in these ecosystems is vital for preserving ecological integrity and understanding potential risks to the human food chain. In this context, fish are considered excellent biomonitoring organisms because they reflect the MP burden in their surroundings through their feeding habits and habitat preferences (Ugwu et al., 2021; De; Jourdan et al., 2024). Although numerous studies have documented MP ingestion across various trophic levels, the vast majority of existing data provide only snapshot representations of current conditions (Khan et al., 2020; Eryaşar et al., 2022; Terzi, 2023; Eryaşar et al., 2025; Eryaşar et al., 2024, 2026), lacking a long-term perspective.

A significant knowledge gap in microplastic research is the scarcity of retrospective data required to establish historical trends and long-term pollution trajectories. Most studies focus on the last decade, obscuring our understanding of the long-term impacts since the global surge in plastic production began. In this regard, museum specimens and university collections serve as invaluable time capsules, offering a unique opportunity to fill these chronological data gaps. Retrospective analyses of museum material allow researchers to pinpoint the onset of plastic contamination and quantify its rate of increase over several decades (Toner & Midway, 2021; Hou et al., 2021; Ilechukwu et al., 2023).

In the present study, 12 *Barbus* species (Daudin, 1805) collected between 2004 and 2018 from five major hydrological basins in Türkiye (Black Sea, Aegean, Marmara, Caspian Sea, and Persian Gulf basins) and preserved in the Recep Tayyip Erdogan University Zoology Collection of the Faculty of Fisheries, Rize, were examined. The genus *Barbus* was specifically selected due to the predominantly benthic (bottom-dwelling) nature of these species. Given that microplastics tend to settle and accumulate in sediments over time, benthic feeding fish are highly reliable indicators of the pollution load within the benthic boundary layer (De Witte et al., 2022).

The primary objectives of this research are (i) to evaluate freshwater MP pollution across different Turkish basins within a broad temporal framework (2004–2018), (ii) to utilize museum specimens to bridge the historical data gap, and (iii) to provide evidence of sediment-to-organism MP transfer through benthic *Barbus* species. This study represents the first comprehensive assessment encompassing all *Barbus* species in Türkiye, offering significant insights at both national and regional scales.

Materials and methods

Sampling and preservation

A total of twelve *Barbus* species were collected from five major basins (Black Sea, Aegean, Marmara, Caspian Sea, and Persian Gulf basins) between 2004 and 2018, representing a wide range of body size and sample numbers (Fig. 1). Despite the broad sampling period, all specimens were preserved under identical conditions to ensure comparability of the data. Fish specimens were sourced from the Aquatic Organisms Collection of the Faculty of Fisheries at Recep Tayyip Erdoğan University, and an evaluation of the existing *Barbus* species in the collection was made.

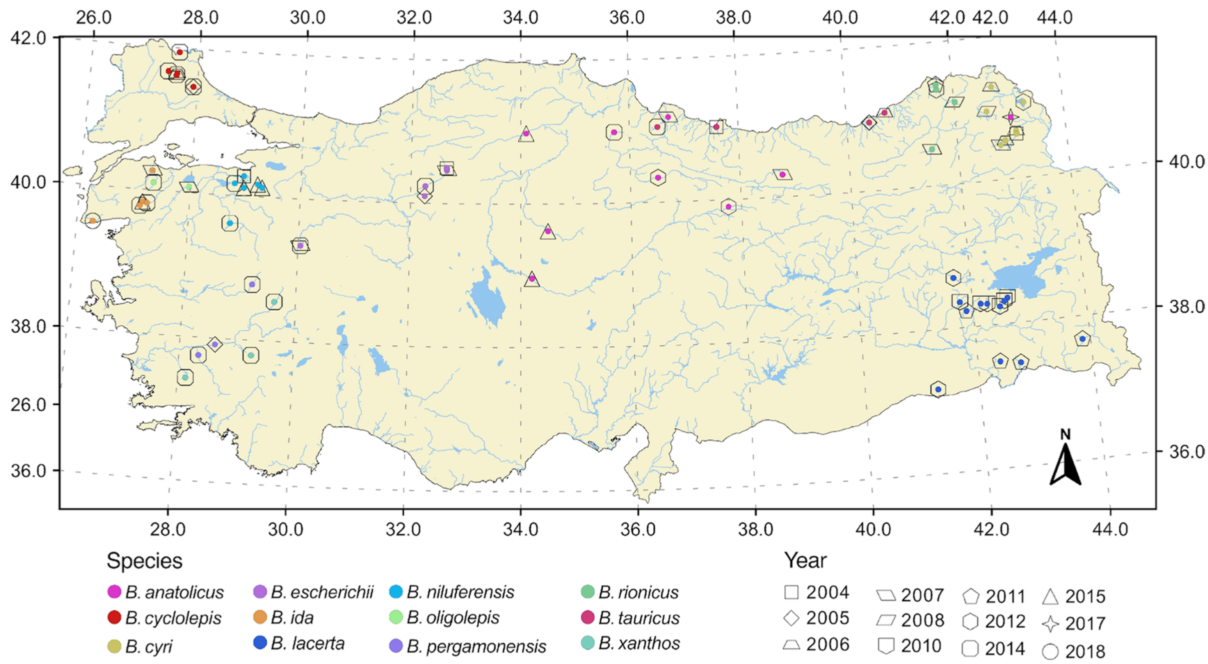

Fig. 1 Geographic distribution of sampling locations across Türkiye. Each symbol represents a distinct sampling site, with marker colors indicating species and marker shapes indicating sampling years

Specimens were obtained through field surveys conducted to supply material to this museum collection. Fish were captured using an electrofishing device in streams and gill nets and cast nets in deeper areas and lakes. Specimens were initially fixed in a 10% formaldehyde solution. In the laboratory, the specimens were then placed in storage containers containing a 5% formaldehyde solution for preservation. The collected specimens were initially separated and identified to species.

Materials and chemicals employed

Hydrogen peroxide (H_2O_2) at 30% concentration was obtained from TEKKIM. Ultra-pure water with a resistivity of 18.2 MΩ cm was used in solution preparation, as well as in washing and rinsing. All solutions and ultra-pure water were filtered through Whatman No. 1 filter paper with an 11 µm particle retention capacity before use. In addition, the glass equipment used was first cleaned and rinsed in an ultrasonic bath. polyethylene (PE), polyethylene terephthalate (PET), and polypropylene (PP) particles, evaluated as test materials in the size range of 150–212 µm, were obtained from basic plastic materials using the manual separation method (Pehlivan & Gedik, 2021, 2022). Furthermore, the extraction success required to achieve the highest recovery was examined, and the most suitable extraction conditions were quantitatively determined.

The process of sample preparation

The methods presented by Gedik et al. (2024) were used as the basis for sample preparation. The fish samples removed from the jars were first rinsed with filtered pure water to remove formaldehyde residues. Then the body weight of each individual was determined using a Shimadzu balance with a sensitivity of ± 0.001 g. It should be noted that long-term preservation in formaldehyde can lead to minor deviations in the biometric measurements (length and weight) of fish specimens due to tissue shrinkage or changes in osmotic balance. However, since all specimens in this study were preserved under identical conditions (initial 10% fixation followed by 5% storage), the potential for preservation-induced bias in the comparative analysis among species and basins was minimized. Length measurements were performed using a Fisherbrand digital caliper providing a sensitivity of 0.01 cm. Following the measurements, a thorough wash was performed to remove any potential contaminants from the outer surfaces of the samples. Digestive system dissection was performed under a laminar flow chamber (Microtest, Türkiye) using stainless steel dissection equipment. To account for potential background contamination, procedural blanks without fish tissue were processed alongside the samples. Each digestive system sample was transferred separately into a 500-ml beaker, and 100 ml of 30% hydrogen peroxide was added. The beakers were then covered with aluminum disks from Isolab (Germany). Digestion was performed in a shaking water bath (Witeg, Germany) at 60 °C for 48–72 h until no visible organic residue remained. To account for tissue hardening caused by formaldehyde fixation, the digestion period in 30% H_2O_2 was extended to ensure complete dissolution of organic matter. Samples that had undergone the digestion process and were completely dissolved were filtered through Whatman No. 1 filter papers with a pore diameter of 11 µm using a glass vacuum filtration system. The filters were then transferred to glass petri dishes, dried at room temperature, and stored for subsequent analysis.

Instrumental analysis for composition, structure, and properties

Particles suspected of being MP were carefully separated using a needle, then imaged and recorded using a RoHS A59.4910 glass digital camera. In the next step, these particles were examined in detail under a stereo microscope (Kyowa SE-L, Japan). The obtained images were analyzed using ImageJ software (Version 1.53a; Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, Maryland, USA) to calculate particle sizes. Subsequently, the particles were evaluated in three separate groups based on their morphological characteristics: fiber, segment, and film (Hidalgo-Ruz et al., 2012).

To check the polymeric structures of the suspected microplastic (MP) particles, all samples (126) were analyzed using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR; PerkinElmer Spectrum 100, Connecticut, USA). Polymer types were identified using measurements in the 4000–650 cm^{-1} spectral range, with a resolution of 4 cm^{-1} and 18 replicate scans. The obtained spectra were compared with reference data in the instrument's library; particles showing at least 70% spectral agreement were classified as microplastics (MP) (Gedik et al., 2023; Eryaşar et al., 2026). The analysis process was limited to particles larger than 100 µm due to difficulties in properly placing small particles on the ATR crystal and limitations in ensuring adequate contact between the infrared beam and the sample (Gedik et al., 2024). A total of 126 particles were isolated from all samples, and 99 of them were identified as MP.

Quality assurance measures

To enhance the reliability of data obtained from microplastic (MP) analyses and to minimize potential sources of contamination, comprehensive quality assurance and quality control (QQ/QC) practices have been implemented (Gedik et al., 2023). All analyses were conducted in a laboratory environment dedicated solely to microplastic studies, and cotton lab coats and polymer-free cotton gloves were used to minimize the risk of personnel contamination. All glassware used in the study was washed in an ultrasonic cleaner, rinsed with filtered deionized water, and protected from external environmental influences by covering it with aluminum foil before use. Potential contamination from long-term storage in formaldehyde was mitigated by rinsing the external surfaces of the specimens three times with ultrapure water before dissection. The formaldehyde solutions used for preservation were randomly sampled and filtered to establish a 'preservation blank,' which confirmed that the storage medium did not significantly contribute to the observed MP counts. Potential contamination and biometric inconsistencies from long-term storage in formaldehyde were addressed by rinsing the external surfaces with ultra-pure water and accounting for potential tissue hardening through extended digestion periods.

A recovery test using spiked PE and PP particles in fixed tissues yielded 95% recovery, validating the efficiency of the modified Gedik et al. (2024) protocol for museum specimens.

Statistical analysis

The normality of the data was tested using the Shapiro–Wilk test. Since the dates were not normally distributed, the Kruskal–Wallis test was used to compare

MP abundance across years, basins, and species. The analysis was performed using the R programming language (ver. 4.5.2). Shapiro–Wilk and Kruskal–Wallis tests were implemented using the rstatix (ver. 0.7.3) package (Kassambara, 2023). The data were visualized using ggplot2 (4.0.0) (Wickham, 2016) and networkD3 (0.4.1). All statistical tests were conducted using a 95% significance level. The data were presented as mean ± standard error (SE).

Results

The number of examined fish was 373. The most represented species were *Barbus lacerta* from the Persian Gulf basin ($n = 81$), *B. cyclolepis* ($n = 47$) from the Aegean Sea basin, and *B. tauricus* ($n = 47$) from the Black Sea basin. The least presented species were *B. cyclolepis* from the Black Sea Basin ($n = 4$) and *B. ida* from the Aegean Sea basin.

Fish length and weight differed considerably among species, ranging from small-bodied taxa such as *B. cyri* (mean length 7.45 cm; mean weight 5.32 g) to larger species such as *B. anatolicus* (mean length 11.69 cm; mean weight 18.93 g). Despite this variation in body size, MP occurrence was widespread across nearly all species and regions (Table 1). Microplastics were detected in 74 (19.8%) of the specimens. The highest frequency of occurrence was observed for *B. xanthos* in the Aegean Sea basin (30.4%), followed by *B. oligolepis* in the Marmara Sea basin (28.6%) and *B. rionicus* in the Black Sea basin (27.8%). On the other hand, *B. cyclolepis* (10.24%), *B. tauricus* (12.8%), and *B. cyri* (16.7%) from the Aegean Sea basin, the Black Sea basin, and the Caspian Sea basin, respectively, had the lowest frequency of occurrence. The samples yielded a total of 99 MP, comprising 29.3% from the Black Sea basin, 22.2% from the Aegean Sea and the Persian Gulf basins, 21.2% from the Marmara Sea basin, and 5.05% from the Caspian Sea basin. When all the data were pooled, *B. ida* (2.00 ± 0.41 MP/ind, mean ± SE) showed the highest average MP abundance, followed by *B. cyri* (1.67 ± 0.67 MP/ind) and *B. lacerta* (1.57 ± 0.37 MP/ind). The species with the lowest MP abundance were *B. escherichii* (1 MP/ind), *B. pergamonensis* (1 MP/ind), and *B. niluferensis* (1.12 ± 0.13 MP/ind, mean ± SE). Across all species and basins, the number of microplastics ingested per individual (MP/ind) ranged from 0 to 3.0. Table 1 shows the specimens in which MP was detected. *B. ida* from the Aegean Sea basin (3.0 MP/ind) had the highest MP loads, although the sample size was small, as did ($n = 4$). *B. cyri* (1.7 MP/ind) from the Caspian Sea basin, *B. ida* from the Marmara Sea basin (1.7 MP/ind), and *B. lacerta* (1.6 MP/ind) from the Persian Gulf basin. Approximately 80% of the specimens yielded no MP. *B. cyclolepis* and *B. escherichii* (1.0 MP/ind) from the Black Sea basin and *B. pergamonensis* from the Aegean Sea basin were the species with the lowest MP contamination. Statistical analysis revealed no significant differences in microplastic concentrations across the sampled basins ($P > 0.05$). While these results do not indicate a total environmental homogeneity, they suggest that, within the current sampling scale and time frame, the detectable pollutant load remained relatively consistent across the study areas.

The microplastic abundance did not show a significant correlation with year (Spearman's rank correlation, $P > 0.05$). No significant temporal differences were observed (K–W test, $P > 0.05$), despite the highest average values occurring in 2018, 2006, and 2012 (Fig. 2A). The highest average MP abundance was recorded from the specimens collected from the Caspian Sea basin (1.67 ± 0.67 MP) (Fig. 2B). From high to low, the average MP abundances were recorded from the Persian Gulf Basin (1.57 ± 0.37 MP), the Black Sea basin (1.32 ± 0.12 MP), the Marmara Sea Basin (1.24 ± 0.11 MP), and the Aegean Sea basin (1.22 ± 0.13 MP). The Kruskal–Wallis test did not determine a significant difference among the basins ($P > 0.05$). On a species basis, *B. ida* (2 ± 0.408 MP/ind) had the highest average MP abundance, followed by *B. cyri* (1.67 ± 0.67 MP/ind), *B. lacerta* (1.57 ± 0.37 MP/ind), and *B. niluferensis* (1.12 ± 0.13 MP/ind) (Fig. 2C). There was no significant difference among the species in terms of MP abundance (K–W test, $P > 0.05$).

A total of 14 distinct polymers were determined in *Barbus* species (Fig. 3). The most frequently observed polymers were EVA (17.2%), polyamide (PA) (17.2%), and PE (14.1%). Acrylonitrile butadiene styrene (ABS), halogen-free flame-retardant polyamide/nylon (HFFR-PA), rubber, polyamide-ethylene–vinyl acetate (PA-EVA), and polystyrene (PS) were the least common polymers, each accounting for 1% of the total composition. In terms of shape, fibers (81.8%) dominated the composition; the remainder

Table 1 Species, sample size (*n*), and descriptive statistics (length and weight) of specimens collected from different basins. Values are presented as mean ± standard error of the mean (SEM)

Basin	Species	*n*	Length range (cm)	Mean length (cm)	Weight range (g)	Mean weight (g)	[1]Fish-MP	[2]FO (%)	[3]MP (*n*)	[4]MP/ind
Aegean Sea Basin	*Barbus cyclolepis* (Heckel, 1837)	48	4.5–10.3	7.19 ± 0.23	2.01–13.23	5.92 ± 0.42	5	10.4	6	1.2
Aegean Sea Basin	*Barbus ida* (Karaman, 1971)	4	13.5–14.5	14.18 ± 0.23	32.1–40.3	37.13 ± 1.91	1	25.0	3	3.0
Aegean Sea Basin	*Barbus pergamonensis* (Kara-man, 1971)	20	8.5–12.7	10.96 ± 0.28	5.23–23.87	15.07 ± 1.16	5	25.0	5	1.0
Aegean Sea Basin	*Barbus xanthos* Güçlü, Kalayci, Küçük & Turan, 2020	23	6.8–16	10.7 ± 0.4	4–23.68	13.18 ± 0.98	7	30.4	8	1.1
Black Sea Basin	*Barbus anatolicus* Turan, Kaya, Geiger & Freyhof, 2018	22	6.5–16	11.69 ± 0.55	3.31–40.43	18.93 ± 2.36	6	27.3	9	1.5
Black Sea Basin	*Barbus cyclolepisv* (Heckel, 1837)	4	7.1–8	7.47 ± 0.19	6.25–7.08	6.58 ± 0.18	1	25.0	1	1.0
Black Sea Basin	*Barbus escherichii* Steindach-ner, 1897	18	6.7–14.9	10.79 ± 0.53	3.86–40.4	15.34 ± 2.11	4	22.2	4	1.0
Black Sea Basin	*Barbus rionicus* (Kamensky, 1899)	18	5.5–18	12.07 ± 0.78	1.7–54.48	22.66 ± 3.64	5	27.8	7	1.4
Black Sea Basin	*Barbus tauricus* (Kessler, 1877)	47	6–15.7	9.71 ± 0.3	2.42–37.75	10.88 ± 0.95	6	12.8	8	1.3
Caspian Sea Basin	*Barbus cyri* (De Filippi, 1865)	18	5–9.6	7.45 ± 0.36	1.59–10.29	5.32 ± 0.64	3	16.7	5	1.7
Marmara Sea Basin	*Barbus ida* Güçlü, Kalayci, Özuluğ, Küçük &Turan, 2021	13	9.3–13	11.23 ± 0.35	9.57–30.23	17.03 ± 1.83	3	23.0	5	1.7
Marmara Sea Basin	*Barbus niluferensis* Turan, Kot-telat & Ekmekçi, 2008	36	4–10.6	8.05 ± 0.22	1.99–18.6	7.69 ± 0.56	8	22.2	9	1.1
Marmara Sea Basin	*Barbus oligolepis* (Battalgil, 1941)	21	6–9.8	8.49 ± 0.24	2.48–10.71	6.96 ± 0.54	6	28.6	7	1.1
Persian Gulf Basin	*Barbus lacerta* (Heckel, 1843)	81	5–13.5	8.54 ± 0.19	1.39–25.47	7.72 ± 0.5	14	17.3	22	1.6

[1]Number of fish with MP in their GIT

[2]Frequency of occurrence

[3]Total number of MP detected

[4]MP per fish (calculated with the fish with MP in their GIT)

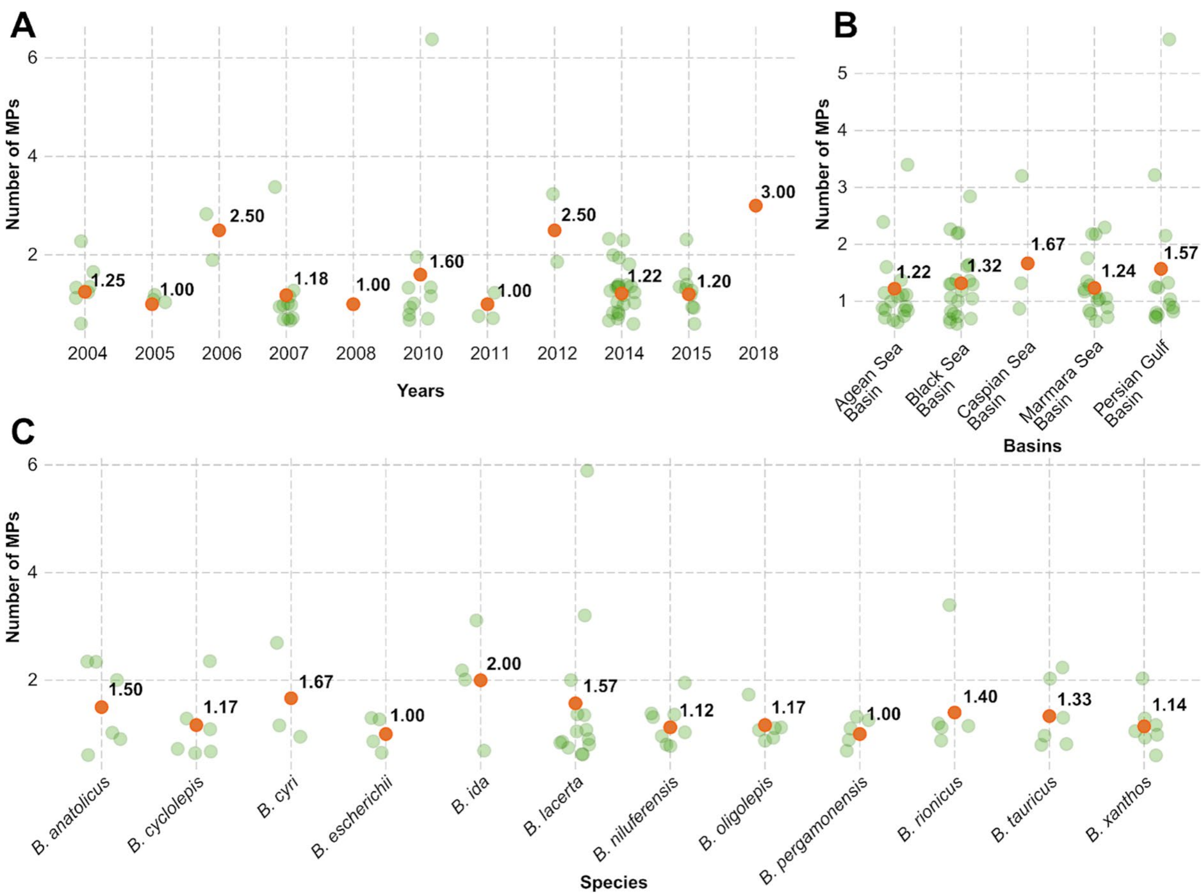

Fig. 2 MP abundance in each specimen by year (**A**), basin (**B**), and species (**C**)

of the MP consisted of fragments (18.2%) (Fig. 3). A total of nine different colors were identified, with black (45.5%) being the most common. The following colors were dark blue (15.2%), red (11.1%), and brown (9.09%) (Fig. 3).

The overall size of the examined MP ranged from 120.0 to 4924 µm, with most particles below 1500 µm and an average of 1253.7 ± 108.7 µm (Fig. 4A). Annual size distributions (Fig. 4B) showed substantial variation within-year and fluctuating mean values; however, no apparent temporal trend was determined (K–W test, $P > 0.05$). The average MP sizes across the basins were broadly comparable, ranging from 1120 to 1463 µm, with extensive overlap among basins (K–W test, $P < 0.05$). Species-specific patterns (Fig. 4D) revealed similarly wide intra-species variation, with moderate differences in mean MP size; *Barbus tauricus* (1621.7 µm) exhibited the largest average particles, whereas species such as *B. cyclolepis* (800 µm) and *B. xanthos* (1105 µm) displayed smaller average sizes. These size variations among the species were not significant (K–W test, $P > 0.05$). Microplastic size was not significantly correlated with fish length or weight (Spearman correlation, $P > 0.05$).

Discussion

This study comprehensively elucidated the presence and distribution of microplastics (MP) in the gastrointestinal tracts (GIT) of *Barbus* species collected from various hydrographic basins across Türkiye. The detection of microplastics in 19.8% (74 individuals) of the 373 examined specimens indicates the extensive prevalence of MP pollution within Türkiye's inland water systems and coastal basins. These findings are congruent with global studies on freshwater

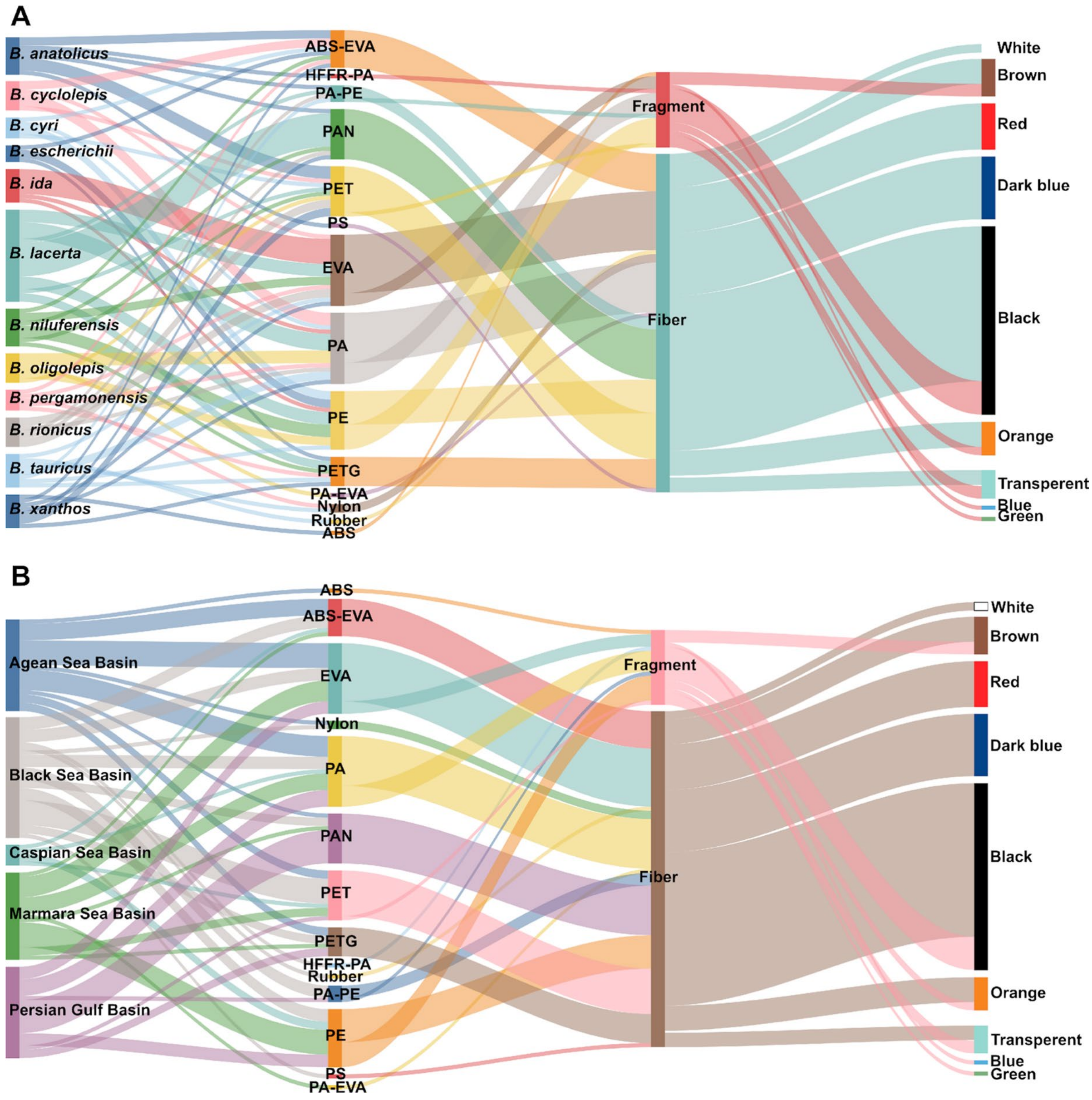

Fig. 3 Sankey diagrams showing the distribution of polymer, shape, and colors by species (**A**) and basins (**B**)

fish (e.g., Jabeen et al., 2017; Parvin et al., 2021) and corroborate that microplastics have become an intrinsic component of aquatic ecosystems. Compared to other studies conducted in Turkish inland waters, the MP occurrence rate observed in this study indicates a moderate level of contamination. For instance, while an 11.8% MP prevalence was reported in mosquitofish sampled from various rivers in Türkiye (Kurtul et al., 2026), the results of this study show a striking similarity to the 20.8% occurrence rate identified in *Squalius* species across 22 basins (331 individuals) (Gedik et al., 2024). This suggests that MP pollution persists at a comparable, chronic level across Türkiye's freshwater ecosystems, irrespective of fish species.

The relatively low MP loads (mean 0.27 MP/individual) and the high proportion of individuals without MPs (~80%) carry significant ecological

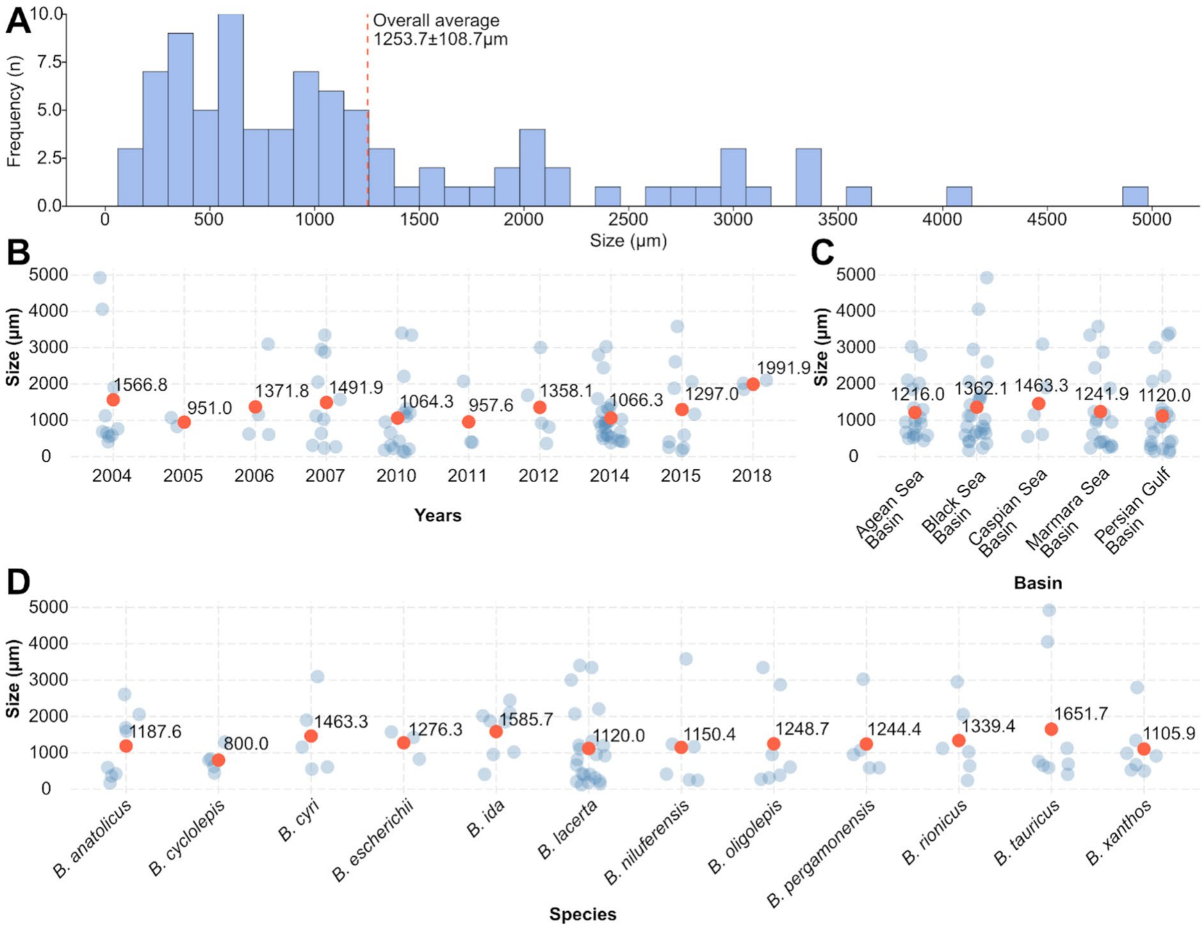

Fig. 4 Overall size distribution of microplastics extracted from *Barbus* species (**A**). Size distribution by year (**B**), basin (**C**), and species (**D**)

implications. These findings likely reflect a dynamic equilibrium between MP intake and egestion capabilities. In an ecological context, this suggests that *Barbus* species may not serve as 'accumulators' that bio-magnify MPs over time, but rather as 'transient indicators' reflecting the immediate, low-intensity environmental flux. This low-level chronic exposure, while not causing physical gut blockage, may still induce sublethal physiological stress or microbiota dysbiosis, suggesting a diffuse pollution scenario in which the impact is characterized by long-term metabolic costs rather than acute physical injury.

Statistical analyses did not detect significant spatial or inter-species variation in MP abundance; however, this outcome must not be interpreted as evidence of equivalent environmental exposure, comparable pollutant pressure, or ecological homogeneity across basins. Under the present sampling design and variance structure, the analyses failed to detect differences and therefore do not, in themselves, demonstrate similar contamination dynamics. Resolving whether the studied basins truly experience comparable MP loading would require higher-resolution sampling combined with direct environmental measurements (e.g., basin-specific sediment and water-column MP concentrations, hydrological flux estimates, and source apportionment analyses), which were beyond the scope of the present retrospective study. Consistent with this caution, the absence of statistical evidence for among-basin variance should not be conflated with the 'evidence of absence' of differential pollution dynamics (Altman and Bland, 1995), and the relatively large within-basin variance observed at some stations is compatible with localized input events that the present design was not powered to detect. Investigations of *Barbus* species from different hydrographic basins reveal that, although MP pollution exhibits regional variation, it is widespread across a broad geographical range. According to the data, the highest mean MP abundance was recorded in the Caspian Sea basin and the Persian Gulf basin, while the Black Sea basin accounted for the largest share of the total MP load (29.3%). However, the absence of a statistically significant difference in mean MP abundance among basins should be interpreted strictly as a failure to detect among-basin differences under the present sampling design rather than as evidence that all regions experience equivalent pollutant pressure. A non-significant comparison of this type cannot, in itself, exclude basin-specific or localized contamination dynamics. A similar lack of detectable among-basin variation has nevertheless been reported in long-term work on *Squalius* species across 22 basins in Türkiye, which also reported no significant variation among basins (Gedik et al., 2024). It must be emphasized, however, that the lack of statistically significant differences in MP abundance among basins does not, by itself, demonstrate that microplastic pollution has become a uniform or saturated environmental stressor across Turkish freshwater systems; it indicates only that the present sampling framework was unable to detect significant among-basin differences. As a hypothetical interpretation that is consistent with the literature but not directly tested in the present dataset, one possibility is that continuous diffuse microplastic input from anthropogenic sources may exceed the natural flushing capacity of individual river systems in heavily impacted regions (Bakir et al., 2014; Horton et al., 2017), leading to a chronic, low-intensity background contamination that would obscure basin-specific differences. Because the present study did not include temporal MP concentration data, sediment contamination measurements, or hydrological transport modelling, such saturation- and footprint-related mechanisms must be regarded as plausible, literature-supported hypotheses rather than as conclusions directly demonstrated by our dataset. The high MP values observed in basins with relatively low population densities (such as the Caspian or Coruh) may be attributed to inadequate wastewater treatment infrastructure and the direct discharge of domestic waste into river systems (Akdemir & Gedik, 2023; Mutlu et al., 2024a, b). The relatively moderate MP levels observed in anthropogenically stressed coastal basins, such as the Marmara and Aegean, could be attributed to the rapid hydrologic transfer of debris from riverine systems to the marine environment. This dynamic may serve as a mitigating factor for long-term riverine accumulation; however, without site-specific flow and transport modeling, this remains a plausible hypothesis warranting further investigation (Gündoğdu et al., 2018; Akdemir & Gedik, 2023). Furthermore, the insignificant difference in polymer types, shapes, and color compositions across basins may be tentatively consistent with the hypothesis that a broadly comparable polymer signature—dominated by fibrous and packaging-related polymers—occurs in the sampled fish across these basins. The absence of statistically significant differences in polymer composition does not, however, confirm equivalent environmental loading or rule out basin-specific source mechanisms operating below the detection sensitivity of this study; direct environmental measurements (e.g., wastewater, sediment, and water-column polymer profiling) would be required to test this interpretation.

Temporal analyses conducted within the scope of this study revealed relative peaks in 2006, 2012, and 2018; however, Spearman correlation analysis showed no significant relationship between MP abundance and years. This is consistent with the large-scale *Squalius* study covering 2004–2018, which also reported no significant change in MP abundance over the 15 years (Gedik et al., 2024). The absence of a significant temporal trend in microplastic abundance across the 15-year study period must be interpreted strictly as a failure to detect a significant change under the present sampling design and variance structure, rather than as evidence that Turkish freshwater basins have reached an ecological saturation threshold for microplastics. The present study did not include temporal environmental concentration measurements, sediment time-series, or hydrological flux estimates, all of which would be required to test such saturation dynamics. As a hypothetical interpretation that should be regarded as speculative until verified by direct environmental monitoring, the stability of MP ingestion in *Barbus* during 2004–2018, despite a rising global production trajectory, may be tentatively consistent with the literature-based hypothesis that, in heavily impacted freshwater regions, diffuse and chronic MP inputs can dominate ingestion patterns more than year-to-year variation in production (Hou et al., 2021; Toner & Midway, 2021). While the literature describes scenarios in which long-term, diffuse plastic input can produce persistent low-level contamination (Bakir et al., 2014), the present dataset is not capable of demonstrating that such a state has been reached in Turkish freshwater systems; this remains a hypothesis to be tested by future environmental monitoring rather than a study-derived conclusion. The fact that approximately 80% of the specimens did not contain MP may be attributable to the fish's egestion capabilities or differences in exposure at the time of sampling (Collard et al., 2019; Horton et al., 2018). Although the Kruskal–Wallis test did not detect statistically significant differences in mean MP abundance among basins, the cumulative numbers of particles recovered per basin must be interpreted with substantial caution. These cumulative percentages are sensitive to uneven sample sizes among basins, unequal species representation, and differences in sampling intensity, and therefore should not be regarded as standardized, per-effort indicators of basin-specific environmental MP loading. Without proportional or effort-standardized analyses, such cumulative values cannot, on their own, support quantitative claims about which basin carries the highest total burden. Nevertheless, when considered together with independent environmental evidence indicating intense plastic inputs to these coastal regions (Terzi et al., 2025; Eryaşar et al., 2024), the cumulative patterns observed here may provide a tentative, hypothesis-generating basis for prioritizing targeted future monitoring, rather than a definitive ranking of basin-level contamination.

The observed lack of statistically significant differences in microplastic (MP) abundance among the 12 *Barbus* species does not, by itself, demonstrate that benthic feeding behavior drives MP exposure more strongly than species-specific selectivity; rather, it indicates that the present design did not have the resolution to separate these two potential drivers. As a hypothetical mechanistic interpretation requiring future verification, one possibility consistent with the literature is that the broadly shared benthic ecological niche of these congeners—characterized by foraging in bottom sediments—may translate into a similar probability of incidental MP ingestion, and that in some Turkish freshwater systems the sediment–water interface may carry a sufficiently high background MP concentration to obscure subtle species-specific differences. Testing this would require paired sediment MP measurements and feeding ecology data, neither of which were collected in this retrospective study. Within these limits, our observations are nonetheless compatible with the broader pattern in which MP uptake in benthic-foraging genera such as *Barbus*.

Polymers with a density higher than that of water tend to settle over time and accumulate in the sediment (Wagner et al., 2014). The typically benthivorous (bottom-feeding) nature of *Barbus* species explains why they are at high risk of MP exposure. Fish with benthic feeding habits experience more direct and continuous exposure to sediment-derived microplastics compared to pelagic species. Numerical variations in MP accumulation observed among the investigated *Barbus* species reflect the impact of the overall ecosystem pollution load on individual species. Analysis of the aggregate data showed that the highest mean MP abundance was recorded in *Barbus ida*, *B. cyri*, and *B. lacerta*. When evaluated by frequency of occurrence, *B. xanthos*, *B. oligolepis*, and *B. rionicus* had the highest contamination rates. However, Kruskal–Wallis test results showed no statistically significant difference in MP abundance among species. This suggests that contamination is related to the common benthic feeding strategy and to environmental MP density, rather than to the biological selectivity of a particular species. However, the lack of significant differences in MP abundance among species and basins limits categorizing *Barbus* as high-resolution biomonitors for spatial mapping. This lack of differentiation may stem from the chronic and widespread nature of MP pollution in Türkiye's freshwater systems, which potentially masks localized point-source variations. While these species effectively reflect the presence of benthic MP, further research is needed to determine whether they can accurately reflect 'fluctuations' in environmental concentrations. Furthermore, the lack of significant correlation between MP abundance or size and fish length or weight suggests that species-based biometric differences do not constitute a determining barrier to MP uptake. Consequently, it can be concluded that species with high frequency values, such as *Barbus xanthos* and *B. oligolepis*, are exposed to microplastics deposited in the sediment due to foraging habits that disturb the bottom sediments. The lack of significant correlation between MP abundance or size and fish length or weight suggests that species-based biometric differences are not primary drivers of MP uptake. Nevertheless, these findings should be interpreted with caution, acknowledging that formaldehyde-induced changes in specimen weight and length over time might slightly mask subtle biometric-pollution relationships.

Among the 14 distinct polymer types identified, EVA, PA, and PE were the most dominant, with EVA widely used in footwear outsoles, packaging, and various industrial foams. The presence of PA and PE polymers—common materials in fishing nets and lines—suggests a possible link to local fishing activities. Although abandoned or illegal fishing gear is known to exist in Turkish inland waters, its specific role as a primary source of secondary microfibers in these basins is inferred from polymer composition rather than direct field observation.

The polymer composition remained consistent across basins, species, and years, although this descriptive pattern, on its own, does not demonstrate that these diverse aquatic systems experience similar pollutant pressures or comparable levels of environmental exposure. Such ecological inferences would require direct measurements of basin-specific MP fluxes, source contributions, and exposure pathways, none of which were undertaken in the present retrospective dataset. The consistency observed here is therefore best described as a uniform polymer signature within the sampled fish across basins; it may be tentatively compatible with widespread anthropogenic influence, but this hypothesis warrants confirmation through higher-resolution sampling and direct environmental quantification. Such findings are consistent with results reported for various fish species sampled from different Turkish inland waters (Gedik et al., 2024; Kurtul et al., 2026). Furthermore, our data align with other investigations conducted in Türkiye's inland water systems (Tavşanoğlu et al., 2020; Gündoğdu et al., 2023; Mutlu et al., 2024a, b). These observations are also supported by global research in freshwater environments, which has identified similar polymer compositions in the digestive tracts of fish (Silva-Cavalcanti et al., 2017; Khan et al., 2020).

The distribution of microplastic shapes in the examined specimens showed a clear predominance of fibers over fragments. The primary factors contributing to the high prevalence of fibers in the gastrointestinal tracts of fish include their ubiquity in their respective habitats, their abundance in consumed prey, and the morphological similarity between these fibers and natural dietary sources (Santini et al., 2022; Santonicola et al., 2023; Gedik et al., 2024). This finding is consistent with numerous global studies conducted on freshwater and marine fish, reflecting the diverse pathways through which fibers enter aquatic ecosystems (Andrady, 2011; Horton & Dixon, 2018). Furthermore, these results are fully consistent with long-term research on Turkish inland waters, such as the 79.1% fiber ratio reported for *Squalius* species (Gedik et al., 2024).

While direct measurements of wastewater effluents were not conducted in this study, the high prevalence of fibers suggests that municipal greywater may serve as a significant vector for microplastic entry into Turkish freshwater systems. Previous reports indicate that a substantial portion of urban wastewater in the region is discharged without tertiary treatment, which potentially contributes to the observed fiber dominance. However, the exact contribution of local infrastructure to the microplastic burden of these basins remains to be empirically quantified. In the context of the study area, previous research on Turkish inland waters has reported that a significant portion of urban wastewater—approximately 50%—is discharged into river systems without tertiary treatment (Akdemir and Gedik, 2023; Gedik et al., 2024). Therefore, the fiber dominance in our samples likely reflects these broader regional wastewater management challenges rather than localized biological selection.

Additionally, fishing tackle, nets, and related gear are recognized as significant sources of fiber-shaped microplastics (Andrady, 2011). Despite regulations, illegal fishing equipment with high fiber content is frequently utilized in Türkiye's inland waters. Due to their low cost, such materials are often abandoned in aquatic environments, where they eventually break down, serving as a continuous source of secondary fibers (Gedik et al., 2024). The marked predominance of black and dark blue fibers (81.8%) is descriptively consistent with a literature-supported hypothesis that inadequately treated domestic greywater—particularly laundry effluents—can act as an important pathway for fiber release into freshwater systems (Browne et al., 2011; Akdemir & Gedik, 2023). However, since direct wastewater sampling was not performed in this study, this remains a literature-supported interpretation rather than a study-derived demonstration of wastewater filtration failure. The ecological implication of this high fiber incidence, combined with the prevalence of dark colors, is a potential 'cryptic dietary shift.' In this scenario, fish energy expenditure in foraging is increasingly diverted toward non-nutritive synthetic materials that mimic natural prey, potentially impacting the long-term physiological fitness of these benthic populations. Consequently, the dense presence of fibers in inland waters appears to be a predictable outcome of these localized anthropogenic activities.

Given the benthivorous feeding strategy of *Barbus* species, it is posited that these fibrous structures—which easily adhere to and settle within the sediment matrix—have a higher probability of accidental ingestion during bottom-feeding compared to fragments. The remaining microplastics identified in this study were fragments. These microplastics originate from secondary sources, resulting from the fragmentation of larger plastic debris due to various environmental stressors (Parker et al., 2021). Fragmented microplastics may stem from materials discarded directly into waterways or transported via runoff; notably, Erüz et al. (2023) reported the direct dumping of plastic waste into riverine systems. Furthermore, the presence of fragment-shaped microplastics in discharge waters has been documented in previous regional studies (Gündoğdu et al., 2018; Akdemir & Gedik, 2023).

Among the identified microplastic colors, black was the most prevalent, followed by dark blue and red. This distribution suggests that these particles may be mistaken for natural food sources due to visual mimicry. The literature indicates that certain fish species exhibit a predatory bias toward dark-colored particles, perceiving them as potential prey items (Ugwu et al., 2021; Aunurohim et al., 2024). However, the predominance of black and dark blue colors may also be directly linked to the environmental abundance of synthetic fibers—particularly from textile products—in these specific hues. The findings of this study are consistent with previous research on both freshwater (Terzi, 2023; Gedik et al., 2024; Kurtul et al., 2026) and marine fish (Eryaşar et al., 2022; 2024) in Türkiye, which also reported a high frequency of black microplastics.

The sizes of the detected MP demonstrate the broad spectrum of particle sizes ingestible by the fish. According to the Kruskal–Wallis test results, the absence of significant differences in MP sizes across years and species suggests that particle-size uptake is constrained by the environmental particle-size spectrum rather than by species-specific selectivity. The lack of a statistically significant difference in MP size distributions among basins suggests that the final size spectrum available for fish ingestion is relatively uniform across the studied hydrographic regions. This uniformity in size characteristics does not necessarily imply identical fragmentation mechanisms; rather, it suggests that despite potential differences in basin-specific environmental stressors, the resulting microplastic profiles reach a consistent size range before biological uptake. Such findings indicate that the environmental 'filter' that dictates which particle sizes are available to benthic foragers operates similarly across these diverse aquatic systems. Furthermore, the observation that MP size does not correlate with fish length or weight indicates that even smaller individuals are susceptible to ingesting relatively large particles, confirming that biometric characteristics do not constitute a definitive barrier to MP uptake. These results are in close agreement with previous studies conducted in Turkish inland waters (Gedik et al., 2024; Kurtul et al., 2026).

The apparent lack of spatial differentiation in microplastic pollution across diverse Turkish hydrological basins carries significant ecological implications. For benthic feeding *Barbus* species, this pervasive background contamination ensures constant exposure, with the high prevalence of wastewater-derived fibers suggesting a steady influx from domestic sources that bypass traditional filtration. This continuous intake, driven by non-selective feeding mechanisms and passive inhalation during respiration, can lead to chronic sublethal effects that extend beyond individual physical injury (Li et al. 2021; Ullah et al. 2025). Ingested particles may cause mechanical blockages, intestinal injury, and microbiota dysbiosis, potentially reducing feeding efficiency and overall nutritional uptake (Bhuyan, 2022; Piskula et al., 2023). Beyond physical harm, the "vector effect" poses a substantial chemical threat, as microplastics can adsorb persistent organic pollutants and leach hazardous industrial additives, leading to oxidative stress, endocrine disruption, and metabolic dysfunctions (Ullah et al., 2025). Over time, these physiological stressors may impair critical behaviors, such as predator avoidance and foraging success, and negatively affect vital rates, including growth, fecundity, and survival (Barboza et al., 2018; Luo et al., 2021; Bhuyan, 2022).

Conclusion

This study provides the first comprehensive assessment of microplastic pollution across all *Barbus* species in Türkiye, using a unique 15-year spatiotemporal dataset. The integration of museum specimens has allowed us to overcome the snapshot limitation of contemporary studies. Although the study spans a period of significant global plastic growth (2004–2018), our data demonstrate a persistent and stable presence of microplastics in Turkish inland waters, with no statistically significant annual increase. The absence of statistically significant spatial and temporal variation must be interpreted strictly as a failure to detect significant differences under the present sampling design, and not as evidence of ecological saturation, a standardized anthropogenic footprint, or a plateaued microplastic influx. Whether Turkish freshwater basins have actually experienced chronic, comparable MP pressure over the past two decades remains a working hypothesis that can only be tested by direct environmental monitoring, including time-resolved sediment and water-column MP measurements and source apportionment analyses. Within these constraints, the benthic life history of *Barbus* species nevertheless makes the genus a useful candidate for monitoring sediment-associated MP. However, the inability of the present design to distinguish among-basin signals indicates that, at the current sampling resolution, these species cannot be regarded as high-resolution indicators of basin-specific contamination intensity. The high prevalence of fibers and specific polymers, such as EVA and PA, indicates that municipal wastewater and the secondary degradation of larger plastic debris are the primary contributors to MP pollution in Turkish freshwater basins. Although mean MP abundances differed among basins descriptively, these differences were not statistically significant under the present design; basin-specific ecological-risk inferences therefore remain tentative and require confirmation through targeted environmental monitoring. The use of archival biological materials is a robust and necessary approach for understanding the long-term trajectory of environmental contaminants. The baseline data established here are crucial for policymakers to develop basin-specific management plans and monitor the effectiveness of future plastic reduction strategies. Future research should focus on the physiological impacts of these accumulated microplastics on fish health and the potential for trophic transfer to higher consumers, including humans.

Acknowledgements This work was supported by the Scientific Research Project Coordination Unit of Recep Tayyip Erdogan University (project no: FBA-2025-1908). We are sincerely grateful to the anonymous reviewers, whose careful and constructive comments substantially improved both the analytical rigor and the clarity of this manuscript.

Author contributions All authors contributed to the conceptualization and study design. Kenan Gedik analyzed data and drafted the manuscript. All authors contributed to revising the final product and subsequently approved its submission.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This study was funded by Recep Tayyip Erdogan Üniversitesi, FBA-2025-1908, Esra Bayçelebi.

Data availability Data will be made available on request.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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