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Microplastic footprints in marsh frogs (*Pelophylax ridibundus*) from two Turkish lakes

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Abstract

Microplastic (MP) pollution poses a growing threat to freshwater ecosystems with potential consequences for inhabiting species such as amphibians. In this study, MP contamination in biotic (*Pelophylax ridibundus*—gastrointestinal tracts) and abiotic (water, sediment) samples from two sampling sites (Büyükçekmece Lake—high human activity, Şavşat Karagöl Lake- low human activity) was investigated between June and August 2024. Water and sediment samples were collected from 10 stations with 15 adult frogs from each site. MPs were detected in 16.6% of frog gastrointestinal tracts (GITs), with fibers dominating (80%) over fragments (20%), and an average of 0.2 MP^{-1} in Büyükçekmece population and 0.13 MP^{-1} in Şavşat Karagöl population. In the environment, MPs occur in 20% of water samples and 30% of sediment samples, with polymer types including polyethylene terephthalate (PET, 29%), polyethylene (PE, 29%), ethylene vinyl acetate (EVA, 21%), polypropylene (PP, 14%), and polytetrafluoroethylene (PTFE, 7%). Büyükçekmece Lake showed higher sediment MP prevalence (40%) than water (10%), while Şavşat Karagöl had more MPs in water (30%) than in sediment (20%), reflecting site-specific dynamics. No significant differences were found between water and sediment MP concentrations. These findings highlight MP occurrence into both the biotic and abiotic components of these ecosystems, with implications for amphibian health and freshwater quality, particularly in areas affected by human activity.

Highlights

- Microplastics were found in 16.6% of marsh frog GITs in two Turkish lakes.
- Fragments (53%) slightly outnumber fibers (47%) when considering all matrices, including biota, water, and sediments.
- PET (42%) is the most common polymers in samples.
- No significant difference was found between water and sediment MP concentrations.
- Study highlights MP risks to amphibians and freshwater ecosystems.

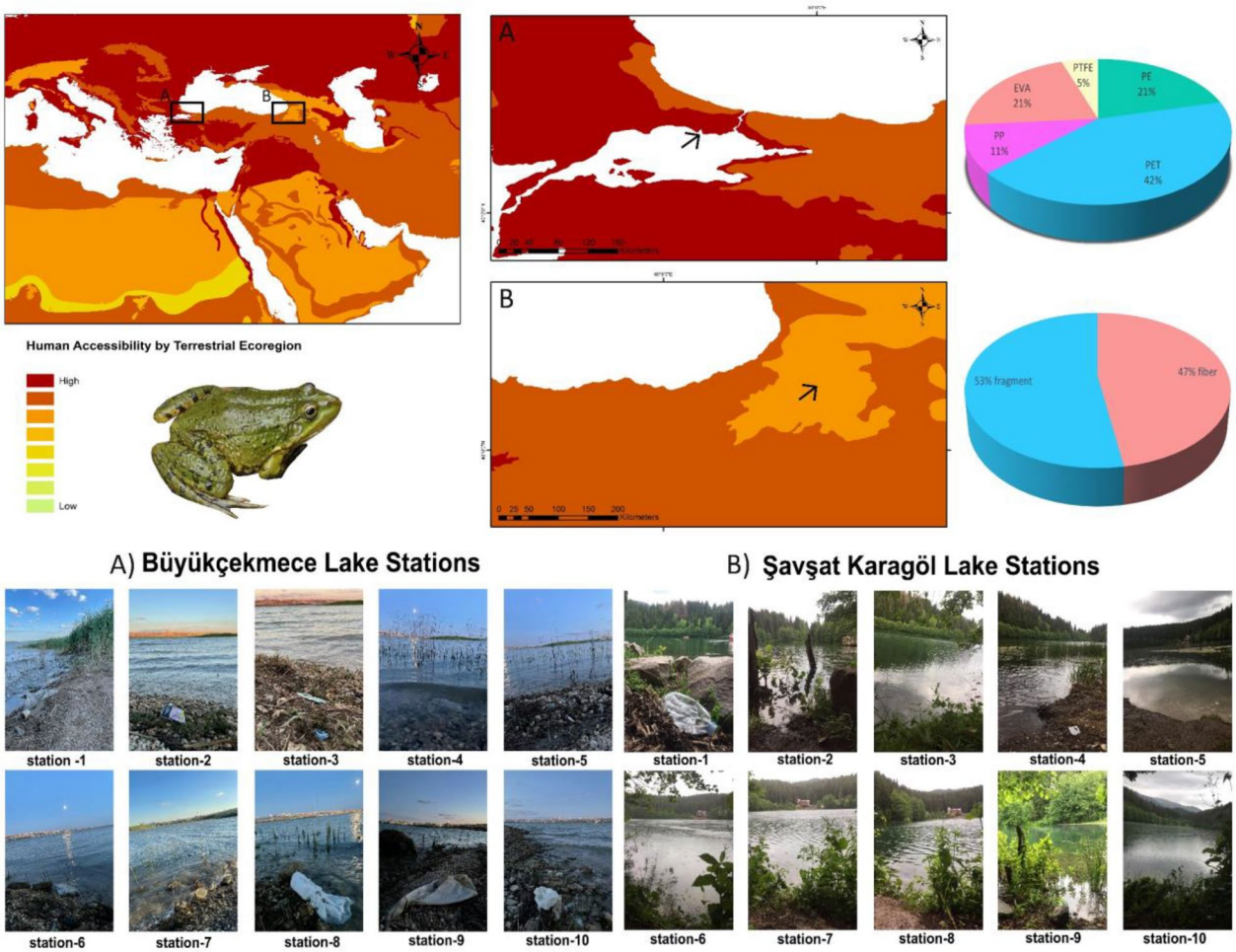
Keywords Bioaccumulation, Environmental contamination, Freshwater ecosystems, Pollution, Sediment

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Graphical abstract



Introduction

Plastics, among the most widely used synthetic polymers globally, have seen exponential growth in production since the 1950 s, reaching approximately 400 million tons by 2024 [39]. Projections estimate that by 2050, global production could soar to 1.1 billion tons if current trends persist [23]. A significant portion of plastics, especially single-use items, has limited recyclability (< 10%), contributing to massive plastic waste generation and widespread pollution [11, 12, 45]. In the environment, plastics undergo degradation through exposure to sunlight, oxygen, and mechanical forces, breaking down into smaller fragments known as microplastics (MPs), typically defined as particles ranging from 1 µm to 5 mm [8, 25]. MPs are highly persistent, persist in ecosystems for hundreds or even thousands of years, and can be transported across vast distances via soil erosion, water currents, and air [4, 17]. Once in the environment, MPs infiltrate living ecosystems, where they are often ingested by organisms that mistake them for food, leading to widespread ecological impacts [3, 6, 44, 54]. Globally, approximately 700 aquatic species, including sea turtles, penguins, and crustaceans, are negatively affected by MP pollution, with effects ranging from physical blockage of the digestive system to reduced reproductive success and altered behavior [19, 22, 55]. Additionally, MPs can induce oxidative stress, inflammation, and changes in gene expression, further compounding their detrimental effects on wildlife [1, 27, 53, 56].

Amphibians, such as the Marsh frog (*Pelophylax ridibundus*), are particularly susceptible to MP pollution because of their biphasic life cycles [14, 33, 48], which span aquatic and terrestrial habitats, permeable skin, and varied feeding behaviors [5, 18, 50]. These

characteristics expose them to MPs via ingestion, water absorption, and contact with contaminated sediments [52]. Recent research has confirmed MP accumulation in amphibian tissues, with studies showing that fibers, often originating from synthetic textiles and consumer products, are the predominant form detected [15]. These particles can act as vectors for heavy metals and other pollutants, potentially amplifying toxicity through bioaccumulation and biomagnification within the food web (Rochman et al., 2013; [29]). Moreover, evidence suggests that MP characteristics, such as fiber length, may vary with frog size, with larger individuals exhibiting shorter fibers, possibly because of in situ biodegradation, which underscores the complexity of MP interactions in amphibian physiology [15].

This study investigated MP contamination in marsh frogs and their habitats across two Turkish lakes with differing human activity levels. We hypothesized that Büyükçekmece Lake, with higher human activity, would have more MPs in water, sediment, and frog gastrointestinal tract (GIT) samples than Şavşat Karagöl Lake. This could contribute to broader ecological challenges for the Marsh frog population, such as altered feeding dynamics, habitat degradation, and increased exposure to contaminants, compared to the less impacted lake.

Materials and Methods

Sampling

In this study, water and sediment samples, along with specimens of the marsh frog (*Pelophylax ridibundus*), were collected from two distinct habitats: Şavşat Karagöl in Artvin, Türkiye, and Büyükçekmece Lake in İstanbul, Türkiye (Figs. 1–2). These locations were selected because of differing levels of human influence, with Lake Büyükçekmece experiencing high tourist activity and Şavşat Karagöl experiencing minimal human presence. Fifteen adult marsh frogs, 10 water samples, and 10 sediment samples were collected from each location, resulting in 30 adult frogs, 20 water samples, and 20 sediment samples from both sites (Fig. 3). The present study was conducted between June and August 2024 to assess the presence and characteristics of MPs in these environments. During the sampling period at Lake Büyükçekmece, 902 mm of average precipitation was recorded, the average daily air temperature was 24.46 °C, the mean UV Index was 2.20, and the mean wind speed was 3.9 m s^{-1}. On the other hand, at Şavşat Karagöl between June and August 2024, the average daily air temperature was 16.5 °C and total precipitation amounted to 1,101 mm, the mean UV Index was 2.06 and the mean wind speed was 2.66 m s^{-1} (NASA [37]). *P. ridibundus* is the species with

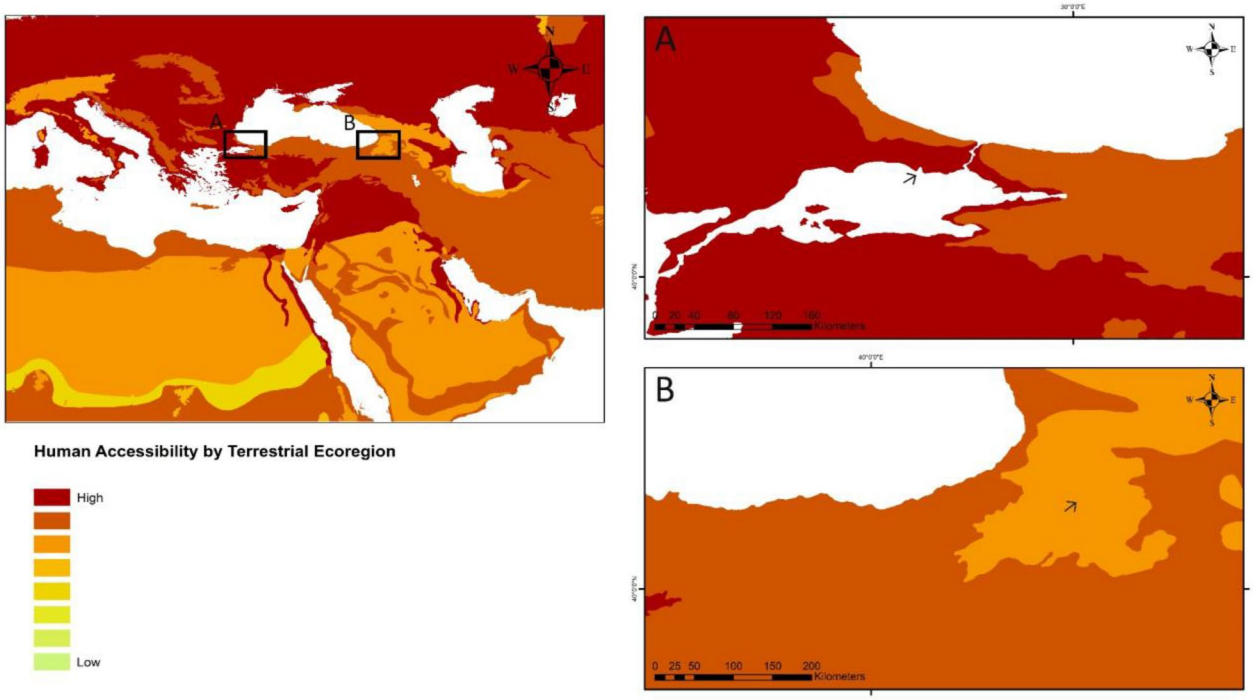


Fig. 1 Human Accessibility by terrestrial ecoregion, **A** Büyükçekmece Lake and **B** Şavşat Karagöl Lake. The black boxes (**A** and **B**) and arrows indicate the location of the lakes studied

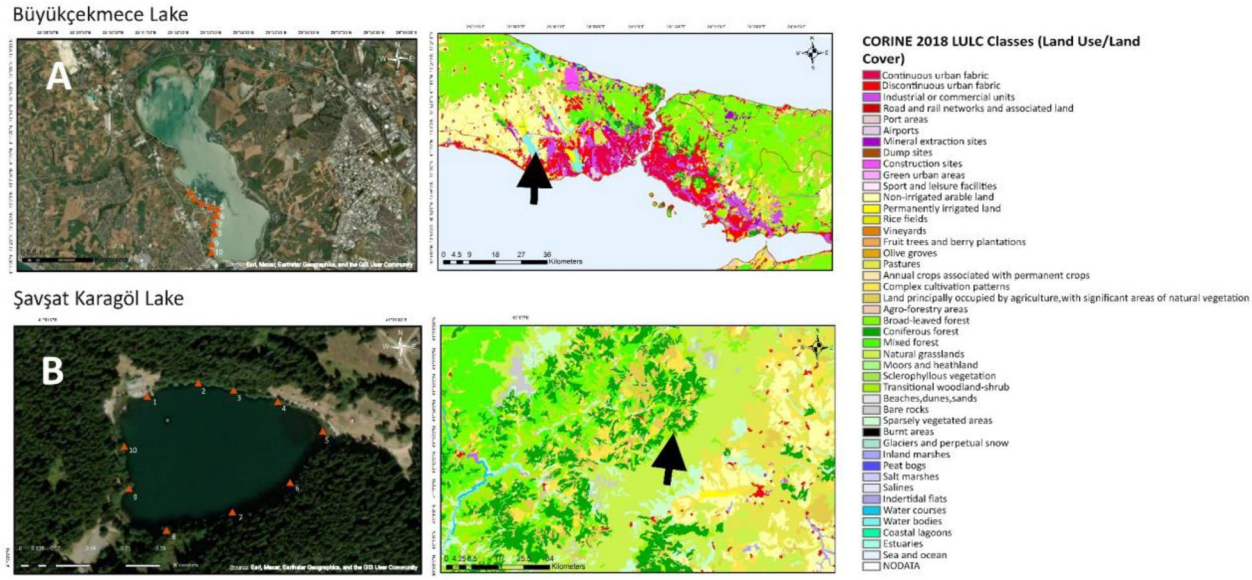


Fig. 2 Imagery land corine maps of **A** Büyükçekmece and **B** Şavşat Karagöl Lakes

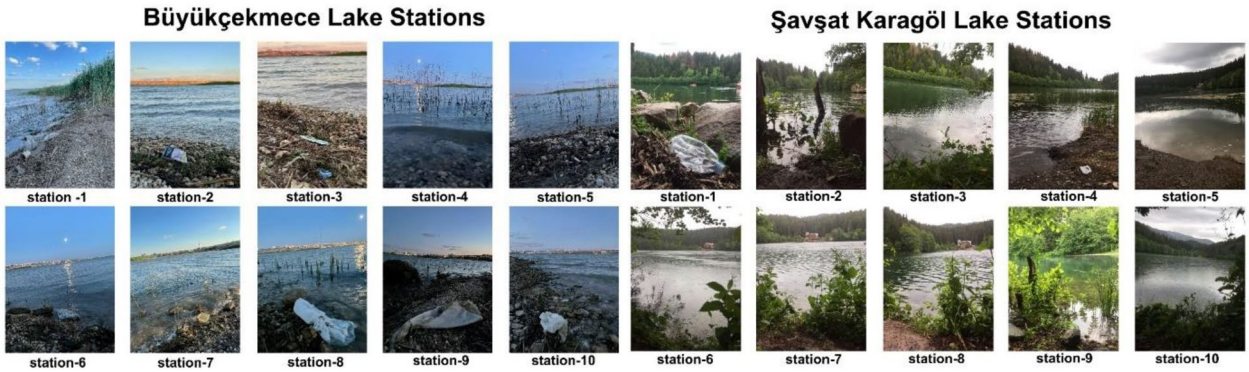


Fig. 3 Photos of stations in Büyükçekmece and Şavşat Karagöl lakes

the broadest distribution in all parts of Türkiye except the Aegean and Mediterranean regions. It is also distributed in Europe and East Asia. The species is listed as Least Concern by IUCN since 2021 [31]. Species identification was based on external morphological characteristics such as scalation, body measurements, and patterning, following the keys and diagnostic descriptions provided by [10]. Frogs were captured manually or with dip nets (Tatlı et al., 2024) and were euthanized using MS-222 (Merck, Darmstadt, Germany) and stored in glass jars containing 70% ethanol (Merck). Water was collected by hand in 5-L wide-mouth jars, while sediment was obtained from the surface layer (0–3 cm depths) using a steel spade and placed into glass jars.

Materials and Chemicals

Hydrogen peroxide (30% H_2O_2) and ethanol were purchased from Merck (Germany) and TEKKİM (Türkiye, Germany), respectively. Ultrapure water with a resistance exceeding 18 MΩ was used for the solution and washing steps. All solutions and ultrapure water were passed through a Whatman filter paper (GF/C (1.2 µm pore size, 47 mm Ø, Whatman GmbH, Dassel, Germany) before use.

The glassware was washed, subjected to an ultrasonic bath, rinsed thoroughly, and covered with an aluminum foil during preparation. Polyethylene (PE), polypropylene (PP), and polyethylene terephthalate (PET) particles, derived from ground granules ranging from 150 to 212 µm in size, were used to assess extraction efficiency (96.2

± 2.3% for PET, 94.5 ± 2.6% for PE, and 98.3 ± 2.9% for PP) and improve recovery outcomes.

Sample Preparation: Adult Frogs

The MP extraction protocol described by Tatlı et al. [51] was used in this study. Frog specimens were rinsed with deionized water (dH_2O), and their snout-vent length (SVL) and body weight (g) were recorded [2, 32]. The gastrointestinal tract (GIT) was removed using a stainless-steel dissection kit and placed in a flask. Then, 150 mL of 30% H_2O_2 was added and the flask was sealed with aluminum foil. The mixture was incubated at 60 °C with shaking at 80 rpm (Lab Companion, Shanghai, China) for three days. After complete digestion, the contents were filtered through Whatman GF/C filters (Borosilicate glass, 1.2 µm pore size, 47 mm Ø), transferred to glass Petri dishes, air-dried at ambient temperature, and stored for subsequent analysis.

Water Samples

Each 5-L water sample from the stations was first sieved through a 45 µm steel mesh (Retsch), and the retained material was carefully moved to a beaker. Fifty milliliters of H_2O_2 were added, and the sample was maintained at 60 °C with 80 rpm agitation for one day to degrade the organic material. Once fully processed, the sample was filtered through Whatman GF/C filters, placed in glass Petri dishes, and kept at room temperature for later examination [51].

Sediment

All sediment samples were thoroughly homogenized using a clean stainless steel spatula in a glass container to ensure uniform distribution of particles before subsampling. Sediment samples were dried in an oven at 45 °C for 5–7 d until their weights stabilized. Subsequently, 100 g of dried sediment (estimated wet weight: 120 g) was placed in a beaker, mixed with 500 mL of saturated $ZnCl_2$ solution (1.65 g $cm^{-}3$) using a glass rod, and left to settle for about four hours. The supernatant was extracted under vacuum using a glass tube and filtered. Material from the supernatant was captured with 25 µm plankton nets, which were rinsed with filtered deionized water and transferred to fresh beakers. This process was repeated three times to maximize MP recovery. To eliminate organic content, 50 mL of 30% H_2O_2 was added, and the beakers were incubated at 60 °C with shaking at 80 rpm for 1–3 days. The samples were then filtered through Whatman GF/C filters, placed in glass Petri dishes, and stored at room temperature [51].

Instrumental Analysis

Visual Examination

All filters were analyzed using a stereomicroscope (Leica S6D, 6.3 ×magnification). Any particles considered to be potential MPs were transferred to a clean filter, and their images were captured (Leica EC3, Leica Microsystems, Switzerland). Additionally, the recorded images were analyzed using the ImageJ software developed by the National Institutes of Health (NIH) (Version 1.53a; Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2014). Once the particle size was determined, the particles were classified into fragments and fibers based on their morphology [26].

Polymer Composition

An attenuated total reflection (ATR) Fourier transform infrared spectrometer (FTIR, PerkinElmer, Spectrum 100) was used to verify the composition of the suspected MPs. Each particle was positioned on the ATR crystal, and spectra were recorded from 4000 to 650 cm^{-1} at a 4 cm^{-1} resolution with 32 scans (see Fig. 4). These spectra were matched against reference polymer profiles, and particles showing at least 70% similarity were confirmed to be MPs [5]. Only particles whose spectra matched known synthetic polymer libraries in the ATR-FTIR analysis were retained as microplastics; all non-polymeric ("false positive") particles were omitted from MP abundance calculations.

Quality Assurance and Control

Throughout the experimental procedure, rigorous precautionary measures were implemented in the laboratory to prevent the presence of MPs. Airflow from ventilation was restricted, lab access was controlled, and work surfaces were meticulously cleaned. The personnel wore cotton laboratory coats and nitrile gloves. To assess potential contamination, negative blank tests (one per sample) were conducted by processing pre-cleaned glass beakers, without any biological or sediment material, using the same procedures, reagents, and laboratory conditions as those applied to the GIT and sediment samples. Microscopic inspections were performed in clean Petri dishes with filtered water to reduce airborne contamination risks, and any MPs found in the blanks were subtracted from the final count. The recovery efficiency of the digestion and separation procedures was tested using three positive control samples (N = 3), each prepared by spiking a known quantity of PE, PP, and PET particles (150–212 µm) into clean water, sediment, and GIT matrices, respectively. These were processed using

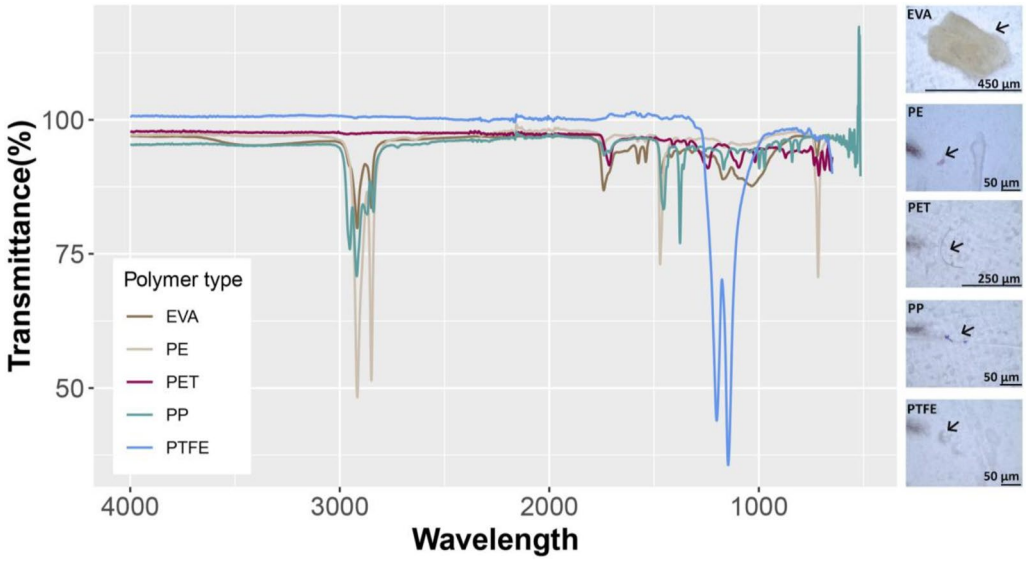


Fig. 4 Fourier transform infrared spectra and stereomicroscope photographs of sampled amphibians and microplastics detected in habitat samples. EVA = ethylene vinyl acetate; PE = polyethylene; PET = Polyethylene Terephthalate; PP = Polypropylene; PTFE = Polytetrafluoroethylen

the same methods as the actual samples [52]. Frog hind leg muscle tissue (0.5 g) was processed following Tatlı et al. [51], treated with H_2O_2, and subjected to the same protocol as regular samples. The initial density separation used 500 mL of $ZnCl_2$ with 100 g of sediment, followed by supernatant extraction. The sediment was then heated at 550 °C for 24 h to remove the organic matter and MPs. Subsequently, pre-prepared PET, PE, and PP particles were mixed into the sediment, and density separation with $ZnCl_2$ filtration was repeated. Particles on filters were counted under a stereomicroscope, and recovery rates were calculated as the ratio of recovered to added particles, yielding 96.2 ± 2.3% for PET, 94.5 ± 2.6% for PE, and 98.3 ± 2.9% for PP. Mean recoveries were 88 ± 3% for surface water samples, 82 ± 4% for sediment samples, and 90 ± 2% for frog GIT homogenates. To assess potential contamination during processing, blank controls (i.e., filters and Petri dishes processed without any environmental sample) were run in parallel with each batch. Blanks underwent the same digestion, filtration, and microscopy steps, and yielded an average of 0.2 ± 0.1 MP particles per blank, demonstrating negligible background contamination.

Data Analysis

The normality of the data distribution for the water and sediment samples was examined using the Shapiro–Wilk test. Owing to the non-normal distribution of the dataset for MPs detected in water samples collected from different locations ($p < 0.05$), the Mann–Whitney U test was applied. All statistical analyses were performed using the Stats Package [41, 42]. Stacked bar charts were created using the ggplot2 package [57] to visualize the frequencies of MPs in subcategories such as water, sediment, and specimens, while violin plots were used to display the distribution of MP sizes. A Chord diagram illustrating the distribution of MPs in terms of shape, color, and polymer type across the samples was generated using a circular package [24]. The data were analyzed using R programming v4.4.2 [41, 42].

Results

Our study highlights the accumulation of MPs in the gastrointestinal tracts of adult marsh frogs, which are frequently used as bioindicators in the literature, as well as in water and sediment samples collected from their habitats in two lakes in Türkiye, Şavşat Karagöl (Artvin) and Büyükçekmece (İstanbul). Pollution parameters that may accumulate in aquatic environments, such as lakes with high accessibility owing to human activity, can affect animals inhabiting these habitats (Fig. 1). These findings suggest that the presence of MPs in these habitats might affect amphibians. The ATR-FTIR device analyzed 90 suspected MP particles, identifying 19 (21%) as synthetic polymers.

Microplastics in Frogs

Overall, MPs were detected in the GITs of adult frogs at a rate of 16.6% (in five out of 30 samples) and were zero in the three combined laboratory/procedural blank samples.

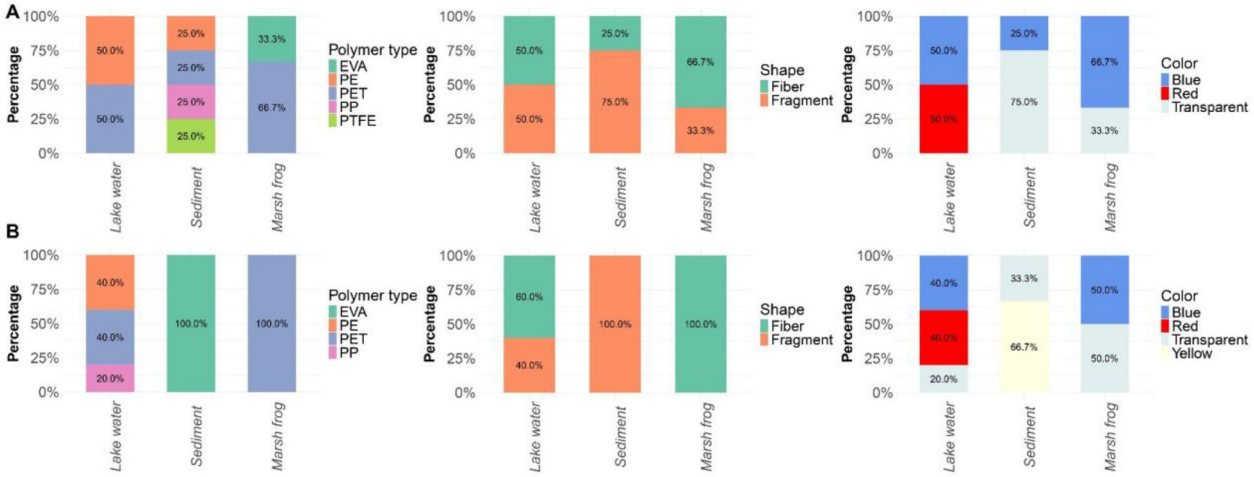


Fig. 5 Stacked bar plots categorizing the microplastics detected in water, sediment, and adult frogs: **A** Büyükçekmece Lake, **B** Şavşat Karagöl Lake

In marsh frog samples from Büyükçekmece Lake, the polymer composition of MPs was 33% ethylene–vinyl acetate (EVA) and 67% PET, with a shape distribution of 67% fibers and 33% fragments, and a color distribution of 67% blue and 33% red (Fig. 5a). In contrast, marsh frog samples from Şavşat Karagöl showed a polymer composition of 100% PET, with all MPs being fibers (100%) and an equal color distribution of 50% blue and 50% transparent (Fig. 5b).

Overall, while 0.2 MP per specimen was found in the Büyükçekmece Lake, 0.13 MP per specimen was found in the Karagöl Lake. GITs from Büyükçekmece Lake exhibited two polymer types (EVA and PET), whereas those from Şavşat Karagöl Lake contained only one polymer type (PET). In the marsh frog samples, fiber-shaped MPs were dominant in Büyükçekmece Lake (66.7%), whereas all MPs in Şavşat Karagöl Lake were fiber-shaped (100%). Of the particles detected in amphibian GITs from the two lakes, 80% were fibers and 20% were fragments (Fig. 6). In marsh frog samples, Büyükçekmece Lake contained blue (66.7%) and transparent (33.3%) MPs, whereas Şavşat Karagöl Lake contained equal proportions of blue (50%) and transparent (50%) MPs. The length of the MPs ranged from a minimum of 246 µm to a maximum of 498 µm, with a mean length of 357.4 ± 44.31 µm (Fig. 7).

Microplastics in Waters and Sediments

In water samples from Büyükçekmece Lake, MPs were detected only at station 8 (mean = 0.2 MP per station), where the MP composition consisted of fragments and fibers, both at 50% each, with polymer types of 50% PE and 50% PET, and a color distribution of 50% blue and 50% red (Table 1). In contrast, lake water samples from Şavşat Karagöl showed MP presence at stations 1, 8, and 10 (mean = 0.5 MP per station). In water samples from Şavşat Karagöl, the polymer composition of MPs was PE (40%), PET (40%), and PP (20%), with a shape distribution of 60% fibers and 40% fragments, and a color distribution of 50% blue and 50% transparent.

In sediment samples from Büyükçekmece Lake, MPs were detected at stations 1, 5, 8, and 10 (mean = 0.4 MP per station)(Table 1). At station 1, fragments composed of transparent PE with a length of 25 µm and a concentration of 0.01 MPs g^{-1}. At station 5, transparent fragments of PP with a length of 43 µm and a concentration of 0.01 MPs g^{-1}. At station 8, fibers composed of PET with a blue color were found, measuring 60 µm in length and a concentration of 0.01 MPs g^{-1}. At station 10, fragments of PTFE with a transparent color were observed with a length of 50 µm and concentration of 0.01 MPs g^{-1}. In contrast, sediment samples from Şavşat Karagöl showed MP presence only at stations 3 and 10 (mean = 0.3 MP per station). At station 3, fragments were the only shapes detected, consisting of 100% EVA with a color distribution of 50% yellow (474 µm) and 50% transparent (83 µm), both at a concentration of 0.02 MPs g^{-1}.

At station 10, fragments composed of yellow EVA with a length of 441 µm and a concentration of 0.01 MPs g^{-1}. The polymer types in lake water were PP (40%), EVA (20%), and PET (40%), whereas PP sediment was the only polymer type detected in the sediment. The shape distribution was 60% fiber and 40% fragment in water, whereas the sediment contained 100% fiber with no fragments detected.

Overall, in the lake water samples, two polymer types (PE and PET) were identified in the Büyükçekmece Lake, whereas three polymer types (PE, PET, and PP) were detected in Şavşat Karagöl Lake. The sediment

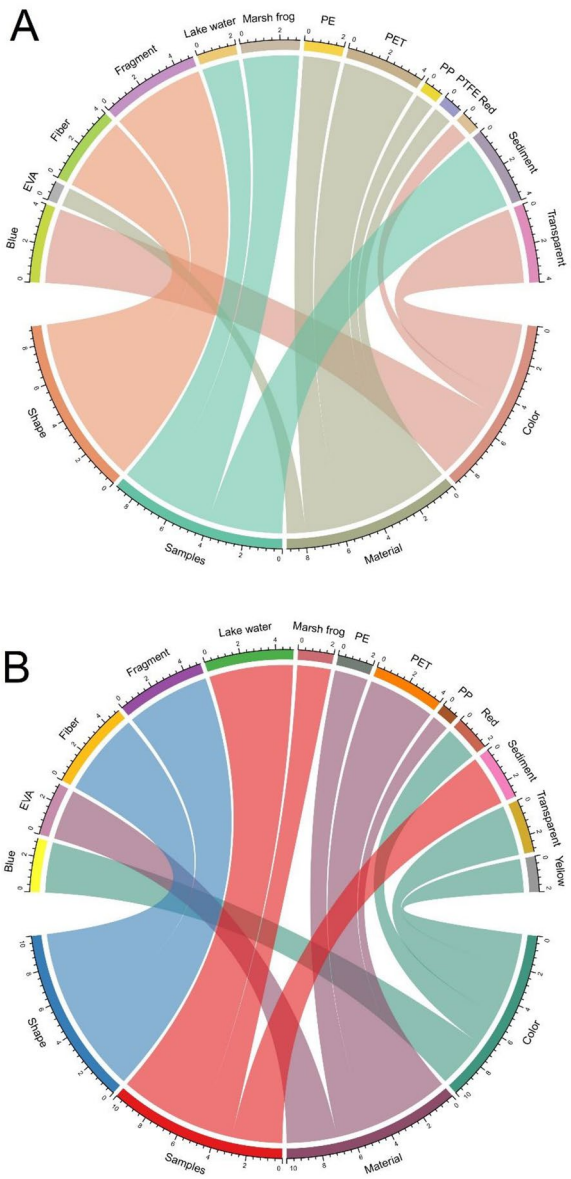


Fig. 6 Chord diagram representing the polymer type, shape, and color of microplastics detected in samples from two habitats: **A** Büyükçekmece Lake, **B** Şavşat Karagöl Lake

samples from Büyükçekmece Lake contained four polymer types (PP, PE, PET, and PTFE), whereas only one polymer type (EVA) was found in Şavşat Karagöl Lake. In the lake water samples, the proportion of fiber-shaped MPs was 50% in Büyükçekmece Lake, whereas it was slightly higher (60%) in Şavşat Karagöl Lake. Sediment samples from Büyükçekmece Lake contained 75% fragment-shaped MPs, whereas all MPs detected in Şavşat Karagöl Lake were fragment-shaped (100%). In Büyükçekmece Lake, the lengths of MPs detected in lake water and sediment samples ranged from a minimum of 25 µm to a maximum of 60 µm (mean: 46.33 ± 4.81). In Şavşat Karagöl, the length of MPs detected in the lake water and sediment samples ranged from a minimum of 45 µm to a maximum of 474 µm (mean: 247.38 ± 66.54) (Fig. 7). The colors observed in the water samples were 40% blue, 20% yellow, and 40% red, whereas 66.7% yellow and 33.3% transparent colors were observed in the sediment samples.

In general, MP was detected in 20% of the water samples and 30% of the sediment samples from all stations. Of the MP detected in the water samples, 58% were fibers and 42% were fragments. In the sediment samples, 86% were fragments and 14% were fibers. Five different polymer compositions were identified in MPs (Fig. 4), with PET being the most common at 42%, followed by EVA at 24%, PE at 21%, PP at 10%, and PTFE at 5%.

According to the Mann–Whitney U test results, no significant difference was detected between the water and sediment samples collected from the two sites (water: p-value = 0.329; $p > 0.05$; sediment: p-value = 0.4795; $p > 0.05$).

Discussion

Microplastics were sporadically detected in the gastrointestinal tract of adult frogs and in the surface water and sediment of both Büyükçekmece and Şavşat Karagöl lakes. Although absolute concentrations were low and most visually-isolated particles did not match synthetic-polymer spectra, these results nevertheless demonstrate that even minimal levels of freshwater plastic pollution can reach higher trophic levels in lake ecosystems.

Microplastics in Frogs

In this study, MPs were detected in 16.6% of adult frog gastrointestinal tracts, with a relatively low detection rate compared to other studies across diverse regions. For instance, Büyükçekmece Lake, located in the megacity of Istanbul with high population density, exhibited an average of 0.2 MP per frog, while Lake Karagöl, in a region with low population density, showed a slightly lower average of 0.13 MP per frog. These findings suggest a potential correlation between human population density and MP ingestion in amphibians, although the detection rates remained lower than those reported elsewhere. Tatlı et al. [52] documented MPs in 44% of sampled frogs across Turkish habitats, whereas Shetu et al. [46] reported a significantly higher detection rate of 90% in frogs from a major delta in Bangladesh. Similarly, Pastorino et al. [40] and Najibzadeh et al. [35] found MPs in 100% and 85.94% of frogs from Italy and Iran, respectively, highlighting the considerable variation in MP ingestion across regions and habitats.

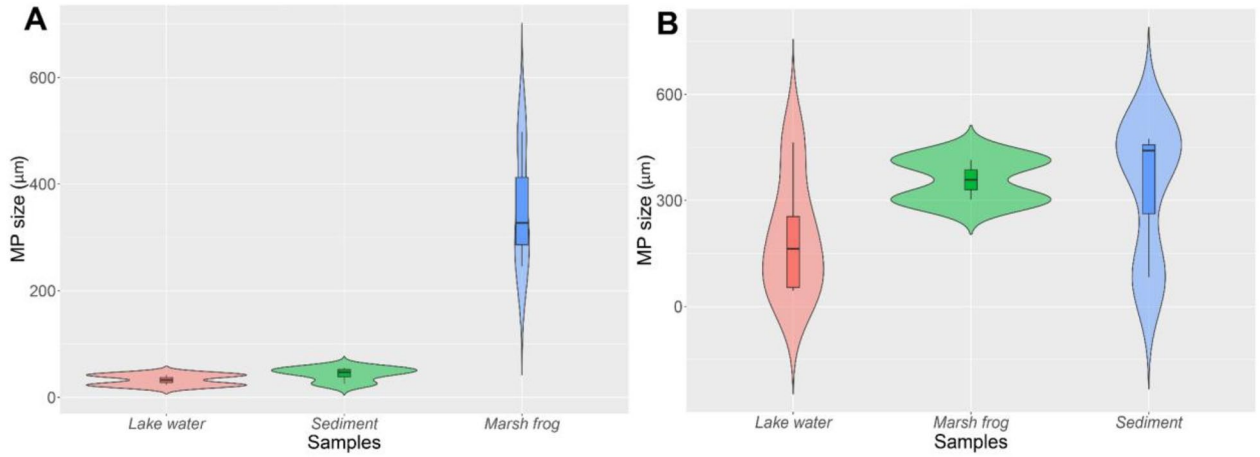


Fig. 7 Violin plot showing the lengths of MPs detected in **A** Büyükçekmece and **B** Şavşat Karagöl Lake

These discrepancies may stem from differences in environmental MP concentrations, feeding behaviors, and habitat contamination levels. Urbanized areas, such as Büyükçekmece Lake, surrounded by industrial and residential activities, are likely to experience higher MP influx through runoff, wastewater discharge, and atmospheric deposition compared to remote areas, such as Lake Karagöl [58]. Frog species with generalist feeding behaviors may also be more prone to incidental MP ingestion, owing to a broader prey range potentially contaminated with MPs [28]. Ecological niche further influences exposure, as Pastorino et al. [40] noted that frogs in lower trophic levels or sediment-rich habitats may ingest MPs more frequently due to proximity to deposition zones. In contrast, Najibzadeh et al. [36] reported higher MP ingestion in aquatic *Pelophylax* sp. (72% of 136 samples) than in terrestrial *Bufotes perrini*, which was attributed to prolonged aquatic exposure and filter-feeding habits.

The predominance of fibers (80%) over fragments (20%) in our study's frog GITs aligns with the global trends in freshwater organisms. Tatlı et al. [52] reported fibers as the dominant MP shape (90.8%) in Turkish frogs, a pattern echoed by Windsor et al. [58], who identified fibers as the most common MP type in freshwater ecosystems worldwide. Najibzadeh et al. [35] similarly found dominant fibers (50.4%) in frog GITs, with polyester (PES) and PET as primary polymers, reflecting textile-derived MPs. In our study, PET prevailed, likely linked to local sources such as fishing gear and packaging materials near Büyükçekmece Lake and Lake Karagöl [13]. The small size, buoyancy, and entanglement of fibers in aquatic vegetation or sediment, where frogs forage, enhances their bioavailability [34, 43, 49].

Although GIT analysis dominates MP studies in amphibians, recent studies have explored alternative exposure pathways. Souza-Ferreira et al. [47] provided in situ evidence of MP exposure via the respiratory and integumentary routes in Amazonian frogs (*Physalaemus ephippifer* and *Boana multifasciata*) and found that the digestive and integumentary routes were the most contaminated, with respiratory exposure being the least significant. This suggests that amphibians face multifaceted exposure risks beyond ingestion, potentially through the skin penetration or inhalation of airborne MPs, particularly in areas with high atmospheric deposition [38]. Such pathways could lead to systemic effects such as epithelial dysfunction or cytotoxicity, as observed in other vertebrates [47].

Burger et al. [15] reported MPs in the tissues (skin, intestine, and corpus) of adult Common River Frogs (*Amietia delalandii*) from South Africa, with 98% fiber-dominated samples (polyester and polyvinyl alcohol). Our study's low GIT detection rate (16.6%) precludes direct tissue comparison; however, the findings of Burger et al. [15] suggest that MPs may persist beyond the GIT, potentially amplifying risks via trophic transfer to predators. The predominance of fibers in both studies (80% here and 98% in [15]) suggests textile and wastewater sources as potential contributors. However, the relatively low MP detection in the urban lake (Büyükçekmece) may be attributed to the absence of direct wastewater treatment plant (WWTP) discharges into the lake. Although urban runoff and atmospheric deposition remain plausible inputs, the lack of direct WWTP effluents likely reduces MP inputs compared to other urban water bodies. In contrast, Şavşat Karagöl is located in a protected natural area with minimal anthropogenic activities, thus further limiting potential MP sources to atmospheric

Table 1 Microplastic characterization in water and sediment samples collected from two lakes in Türkiye

Location	Station number	Samples	MP shape	Polymer type	Color	Length of MP (μm)	MP g^{-1} or L^{-1}
Büyükçekmece Lake	1	Water	–	–	–	–	–
		Sediment	Fragment	PE	Transparent	25	0.01
	2	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	3	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	4	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	5	Water	–	–	–	–	–
		Sediment	Fragment	PP	Transparent	43	0.01
	6	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	7	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	8	Water	Fragment Fiber	PE PET	Blue Red	50 50	0.40 0.40
		Sediment	Fiber	PET	Blue	60	0.01
	9	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	10	Water	–	–	–	–	–
		Sediment	Fragment	PTFE	Transparent	50	0.01
Şavşat Karagöl Lake	1	Water	Fragment	PE	Red	45	0.20
		Sediment	–	–	–	–	–
	2	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	3	Water	–	–	–	–	–
		Sediment	Fragment Fragment	EVA EVA	Yellow Transparent	474 83	0.02 0.02
	4	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	5	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	6	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	7	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	8	Water	Fiber Fiber	PET PET	Red Blue	54 254	0.40 0.40
		Sediment	–	–	–	–	–
	9	Water	–	–	–	–	–
		Sediment	–	–	–	–	–
	10	Water	Fragment Fiber	PE PP	Transparent blue	464 164	0.40 0.40
		Sediment	Fragment	EVA	Yellow	441	0.01

deposition and recreational activities. These contextual differences underscore the need for targeted source-tracking studies to better quantify MP inputs in both urban and rural freshwater systems.

The 16.6% MP detection rate in frogs from Büyükçekmece Lake and Lake Karagöl, primarily consisting of PET fibers, indicates a notable presence of MP pollution in freshwater ecosystems, albeit at a lower prevalence

compared to other regions. This finding aligns with broader patterns of freshwater contamination and suggests potential exposure routes, such as the GIT, respiratory, integumentary, and tissue pathways, as discussed in Souza-Ferreira et al. [47] and Burger et al. [15]. Understanding these pathways and their implications for amphibian health could provide valuable insights into the ecological risks associated with MP contamination, particularly in regions subject to anthropogenic impacts.

Microplastics in Water and Sediments

The investigation of MP pollution in freshwater systems inhabited by marsh frogs has revealed varying levels of contamination across water and sediment samples, with implications for both environmental dynamics and amphibian ecology. In Büyükçekmece Lake, MPs were detected in only 10% of the water samples (1 out of 10 stations) but in 40% of the sediment samples (4 out of 10 stations), suggesting a tendency for MPs to accumulate in sediments rather than remain suspended in the water column. This aligns with the findings of Ballent et al. [9], who attributed the higher sediment MP prevalence to sinking processes driven by the particle density and local hydrodynamics. Conversely, Şavşat Karagöl Lake exhibited a higher MP detection rate in water (30%, 3 of 10 stations) than in sediment (20%, 2 of 10 stations), potentially reflecting recent inputs from surface runoff or tourism rather than long-term sediment accumulation, as suggested by Imhof et al. [30]. These differences underscore the influence of site-specific factors, such as water currents and anthropogenic activity, on the MP distribution.

Comparatively, a broader study by Tatlı et al. [52] across 11 Turkish freshwater sites reported MPs in 36% of both water and sediment samples, indicating moderate but not ubiquitous contamination. While Tatlı et al. [52] noted higher MP concentrations in areas with intense human activity (e.g., Bursa station), the overall detection rates were loosely within the 20–40% range observed in the Büyükçekmece and Şavşat Karagöl lakes, as well as in Lake Garda, Italy [20]. However, the variability in MP abundance—ranging from 0.5 MP per station in Şavşat Karagöl water to 0.3 MP per station in its sediment—highlights localized hotspots rather than uniform contamination, a pattern potentially linked to proximity to human settlements or recreational areas. Interestingly, despite the Şavşat Karagöl Lake being described as experiencing minimal human presence, MPs were still detected in its water, sediment, and biota. This finding suggests the influence of indirect MP transport mechanisms.

Atmospheric deposition is one plausible explanation, as airborne MPs can travel long distances from urban or industrialized regions before settling into remote ecosystems [4]. Additionally, MPs in surrounding soils—potentially originating from historical land use, atmospheric fallout, or the application of contaminated organic fertilizers—may be mobilized by surface runoff or subsurface flow, ultimately reaching aquatic systems [16, 44]. Hydrological connectivity between distant land-based sources and the lake could thus facilitate MP entry, even in areas with limited direct human activity. These findings align with recent literature emphasizing the pervasive nature of MP contamination and the complexity of their transport routes across environmental compartments.

The MP characteristics further illuminated these dynamics. In Büyükçekmece Lake, the water and sediment samples showed an even polymer distribution (water: 25% each of PE, PP, PET, and PTFE; sediment: 50% PE and 50% PET). Şavşat Karagöl Lake water contained PE (40%), PET (40%), and PP (20%), with sediments containing exclusively EVA (100%). The prevalence of PE and PET in sediments across both lakes aligns with Andrady's [7] observations of its density-driven sinking behavior, while the diversity in water samples suggests a mix of buoyant and recently introduced MPs. Shape analysis reinforces this: Fiber-dominated Şavşat Karagöl water (60%), Büyükçekmece water showed an equal distribution of 50% fibers and 50% fragments, and Büyükçekmece sediment contained 25% fibers and 75% fragments, with Şavşat Karagöl sediment consisting entirely of fragments (100%), indicating that smaller, less dense particles may persist in the water column.

Color patterns provide additional clues about MP sources. Büyükçekmece Lake water MPs were 50% blue and 50% red, whereas the sediments were 75% transparent and 25% blue. In Şavşat Karagöl, the water samples were 40% blue, 40% red, and 20% transparent, with 66.7% yellow and 33.3% transparent sediments. These vibrant colors, particularly blue and transparent, echo the anthropogenic signatures (e.g., fishing gear, textiles, and plastic litter) noted by Tatlı et al. [52] in Turkish freshwater, although their study emphasized the presence of blue MPs in water. The shift toward transparent and yellow MPs in Şavşat Karagöl sediments may reflect weathering or different local inputs such as agricultural runoff, warranting further investigation.

Overall, MP detection across both lakes averaged 20% in water and 30% in sediment, with polymer compositions dominated by PET (29%), PE (29%), EVA (21%), PP (14%), and PTFE (7%). These levels are moderate compared to heavily urbanized systems like Lake Ontario (1.1 MP m^{-3}; [9]) but align with less impacted sites like Lake Hovsgol, Mongolia (0.03–0.4 MP m^{-3},[21]). Non-significant differences between water and sediment MP concentrations in Büyükçekmece and Şavşat Karagöl Lakes suggest that a

homogeneous distribution is possibly driven by consistent hydrodynamics or uniform contamination sources. This contrasts with Imhof et al.'s [30] reports of spatial variation elsewhere but mirrors Tatlı et al.'s [52] finding of limited statistical differences across their sites. For marsh frogs, the presence of low-to-moderate MP in these habitats likely influences ingestion rates, positioning them as bioindicators of freshwater contamination. The interplay between environmental factors (e.g., sedimentation and water flow) and anthropogenic pressures (e.g., tourism and urban runoff) driving these patterns merits further study, particularly in small water bodies, where ecological impacts may be amplified.

Conclusion

This study confirms the presence of MPs in the gastrointestinal tracts of adult marsh frogs as well as in the water and sediment of Büyükçekmece and Şavşat Karagöl Lakes, demonstrating the occurrence of plastic pollution into Turkish freshwater ecosystems. The detection of MPs in 16 percent of frog GITs, mostly as fibers, suggests a potential risk of bioaccumulation for amphibians in these habitats. The occurrence of MPs in 20% of the water samples and 30% of the sediment samples indicates localized but detectable environmental contamination, suggesting that while microplastics are present, their distribution is relatively limited across the sampling sites. The differences between the two lakes—higher sediment MP presence in Büyükçekmece and greater water MP occurrence in Şavşat Karagöl—point to the varying influences of local conditions such as human activity and environmental processes. Although MP levels in these lakes are not as high as those in some heavily impacted urban systems, their presence across both biological and environmental components raises concerns about long-term ecological effects. Understanding seasonal changes, the potential movement of MPs through food webs, and the role of lake-specific factors, such as water flow and surrounding land use, is essential. Additionally, exploring how MPs interact with other pollutants could shed light on the broader environmental risks. Efforts to reduce plastic waste and improve local waste management practices remain critical to lessen the impact of MPs on aquatic ecosystems.

Author contributions

H.T. and A.A. wrote the main manuscript text and H.T. prepared Figs. 1–7. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the local ethics committee of Recep Tayyip Erdogan University, which ensured that all specimens were treated ethically and humanely (Decision No: RTEÜ.2024/32).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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