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Distribution of microplastics in tadpoles, adults, and habitats of three water frogs of *Pelophylax* spp.

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Abstract

Microplastics (MPs) are ubiquitous environmental contaminants, and amphibians are especially susceptible owing to their dual aquatic and terrestrial life cycle and permeable integument. This research examines the presence, composition, and potential origins of microplastics in tadpoles, adult frogs, and their environments in different regions of Türkiye, concentrating on three Anatolian water frog species: *Pelophylax ridibundus, P. bedriagae*, and *P. caralitanus*. Samples were obtained from 11 sites, encompassing water bodies, sediments, tadpoles, and the gastrointestinal tracts (GITs) of adult frogs. MPs were examined for polymer type, morphology, and dimensions via stereomicroscopy and Fourier transforms infrared spectroscopy. Results indicated that microplastics were absent in 64% of sediment and water samples; nevertheless, elevated quantities were identified in areas with considerable human activity, such as İstanbul. Tadpoles demonstrated negligible MP intake, with merely 2 MPs identified among 157 samples, resulting in an average of 0.01 MP per individual. Conversely, 44% of the 100 adult frogs exhibited microplastics in their gastrointestinal tracts, averaging 1.52 microplastics per individual. The MPs were primarily fibers (90.8%) and comprised polymers such as ethylene–vinyl acetate (EVA) (32.5%) and polycyclohexylenedimethylene terephthalate (PCT) (20.8%). The study emphasizes the potential dangers of microplastics to amphibian health and reinforces the necessity of addressing microplastic pollution in freshwater habitats through focused conservation initiatives.

Keywords Amphibians, Aquatic animals, Inland water, Microplastic, Pollution

Introduction

Microplastics (MPs), defined as plastic particles ranging between 1 µm and 5 mm in size, have emerged as a significant environmental issue due to their persistence in ecosystems and potential effects on the health of various organisms [1–6]. These MPs are derived from multiple sources, such as the degradation of larger plastic waste, synthetic textiles, and other plastic-based consumer products [7, 8]. Once in the environment, MPs are ingested by aquatic organisms, which can cause harmful effects such as physical blockages, reduced nutrient absorption, and exposure to toxic chemicals adsorbed onto plastic particles [9–11]. This process creates a pathway for bioaccumulation by introducing MPs into the food web and poses risks to higher trophic levels, including fishes, amphibians, reptiles, birds, and mammals [12–17].

The International Union for Conservation of Nature (IUCN) has designated 41% of amphibian species as endangered, representing the most threatened group of vertebrates, with their global population decreased by 70% (IUCN 2024; [18, 19]). The majority of the threatened species are found in riverine and lentic

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environments [20]. Pollutants entering aquatic ecosystems through diffusion or fixed point sources, even at relatively low concentrations, have also been identified as a detrimental factor associated with population declines [21]. Therefore, anthropogenic water pollution is one of the most significant causes of anuran declines, particularly in small water bodies [18]. Additionally, amphibians transfer pollutants through the food web, moving contaminants from aquatic to terrestrial environments. Therefore, the concentrations and effects of pollutants such as heavy metals, pesticides, and others in frog tissues have been the subject of many studies [10, 22–24] .

Amphibians are especially susceptible to MPs due to the permeable nature of their skin, feeding habits, and aquatic and terrestrial life cycles [25, 26]. These factors make amphibians valuable bioindicators for studying environmental pollution [27–31]. Recent studies have detected MPs in several amphibian species worldwide [32–35]; however, data on MPs in amphibians are limited, especially in Türkiye, where several frog populations occupy critical habitats that may be affected by pollution [12, 36]. In particular, Anatolian water frogs (*Pelophylax* spp.), widespread in Türkiye's freshwater ecosystems, provide an opportunity to study the presence, distribution, and species of MPs across different regions and habitats [37]. Among Anatolian water frogs, the marsh frog *P. ridibundus* is the species with the broadest distribution in all parts of Türkiye except the Aegean and Mediterranean regions. It is also distributed in Europe and East Asia. The distribution area of the Levanten frog *P. bedriagae* is relatively narrow, extending from the Aegean and Mediterranean regions of Türkiye to Lebanon and Israel. The Anatolian frog *P. caralitanus* is endemic to Türkiye, and its distribution is limited to the Isparta, Burdur, Denizli, Konya, and Afyonkarahisar provinces, which are collectively defined as the lakes region [38].

Anatolian water frogs (*P. ridibundus, P. bedriagae* and *P. caralitanus*) are likely to accumulate MPs in their gastrointestinal tracts due to their permeable skin and dual aquatic-terrestrial life cycle, with the prevalence and composition of MPs that may be influenced by the level of human activity in their habitats. This research aims to address information deficiencies by examining MP contamination in tadpoles, adult frogs, and their environments (with water and sediment samples) throughout different regions of Türkiye. Our research will enhance the comprehension of microplastic pollution in freshwater ecosystems in Türkiye and guide conservation initiatives to save amphibian populations from environmental pollutants.

Materials and methods

In this study, adult individuals and tadpoles of three species belonging to the genus *Pelophylax* and water and sediment samples taken from their habitats were used. These species are the marsh frog *P. ridibundus,* the Levanten frog *P. bedriagae,* and the Anatolian frog *P. caralitanus.*

Materials and chemicals

Hydrogen peroxide (H_2O_2, 30%) and ethyl alcohol (Merck Darmstadt, Germany) were acquired from TEKKIM (Türkiye) and Merck (Germany), respectively. Ultrapure water with a resistivity greater than 18 MΩ was used for preparing solutions and washing and rinsing. Before use, all solutions and ultrapure water were filtered using a Whatman GF/C filter. Glassware was thoroughly cleaned, maintained in an ultrasonic bath, additionally rinsed, and prepared for use by sealing with aluminum foil. The pre-prepared polyethylene (PE), Polypropylene (PP), and Polyethylene terephthalate (PET) particles, sized between 150 and 212 µm, were sourced from ground granules [39, 40]. These particles underwent detailed analysis to assess extraction efficiency and optimize recovery outcomes.

Sampling

The study involved the examination of adult Anatolian water frogs (*Pelophylax* spp.) and their tadpoles, as well as the collection of sediment and water samples from their natural habitats. Sampling took place between May and September 2022 at 11 different sites, including Adana, Antalya, Burdur, Bursa, Erzincan, Isparta, İstanbul, Kayseri, Manisa, Samsun, and Şanlıurfa (Fig. 1), with habitat images shown in Fig. 2. Sampling stations were determined based on our previous study [37]. Seven of the sampling sites were inhabited by *Pelophylax ridibundus* (sites 1, 2, 7, 8, 9, 10, and 11), two by *Pelophylax bedriagae* (sites 3 and 4), and two by *Pelophylax caralitanus* (sites 5 and 6). A total of 100 adult and 157 tadpole specimens were collected from 11 stations distributed across the range of *Pelophylax* spp. The frogs were captured in various habitats, including lakes, puddles, ponds, and agricultural ditches (Fig. 2), using either manual or dip net methods and then placed in glass jars.

The sites had water bodies of different sizes and features. Detailed information on the sampling sites is given in Table S1. At these stations, frogs are naturally distributed. At the stations, adult frogs were collected using a trap, captured, and euthanized with MS-222 (Merck Darmstadt, Germany), and conserved in containers with 70% ethanol (Merck Darmstadt, Germany). Tadpoles were similarly captured, euthanized, and stored in jars filled with alcohol. Water samples were collected

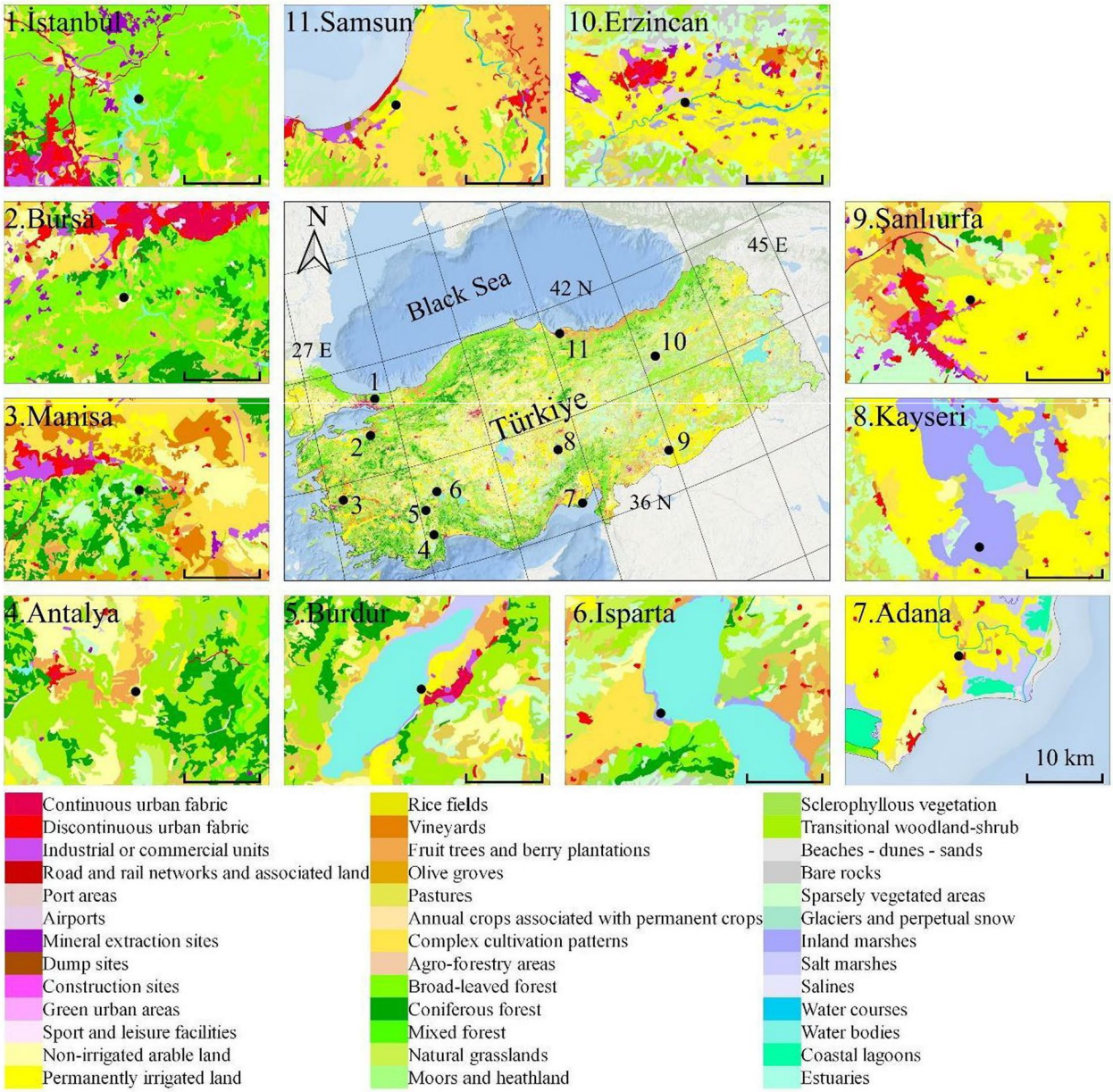


Fig. 1 Station numbers by species. Stations 1, 2, 7, 8, 9, 10, and 11 are the locations where *Pelophylax ridibundus*, 3 and 4 are the locations where *Pelophylax bedriagae* and 5 and 6 are the locations where *Pelophylax caralitanus* samples (adults, tadpoles, water, sediment) were taken [37]

manually using a 5 L wide-mouth jar. Sediment samples were taken from the sediment surface with a steel shovel and bled into a glass jar. Sediment and water samples were collected in three replicates.

Samples preparation

Adult frogs

We followed the MP extraction method outlined by Tatlı et al. [36]. Specimens were cleaned with dH_2O and snout-vent length (SVL)/weight was measured. The gastrointestinal tract (GIT) of specimens was dissected with a stainless steel set and then placed into a flask. Then, 150 mL of 30% H_2O_2 was added to the flask. The flask was sealed with aluminum foil and kept at 60 °C at 80 rpm (Lab Companion, Shanghai, China) for 3 days. The fully dissolved samples were passed through Whatman GF/C filters in a glass filtration system; the filters were set in glass Petri dishes, kept at ambient temperature to air dry, and then stored for subsequent analysis.



Fig. 2 Sampling stations with different habitat types [37]

Tadpoles

The tadpoles collected from each station were placed individually in digestion tubes, 10 mL of H_2O_2 was added and the mouths were covered with aluminum foil. The same procedure was then followed for the adult frogs.

Water samples

The 5 L water sample from each station was first passed through a 45 µm steel sieve (Retsch), and the material collected on it was carefully transferred to the beaker. 50 mL of H_2O_2 was added to each beaker and left for 1 day at 60 °C and 80 rpm to break down the organic matter. The completely dissolved samples were passed through Whatman GF/C filters. The filters were placed in glass Petri dishes and stored at room temperature for analysis [36].

Sediment

Sediment samples were dried (45 °C for 5–7 days) and weighed to constant weight. Then, 100 g of the sediment samples were placed in a beaker and 500 mL of supersaturated $ZnCl_2$ solution (1.65 g cm^{-3}) was added and mixed with a glass rod. After the sediments had settled (approximately four hours), the supernatant was collected by vacuum using a glass tube and passed directly through the filtration system. The materials in the supernatant were collected in 25 µm plankton nets. The plankton nets were rinsed with filtered DI water to remove particles from the net and transferred to new beakers. The whole procedure was repeated three times using the original sediment sample beaker to improve the extraction of MPs from the samples. To remove organic matter from the materials in the beaker, 50 mL (30%) H_2O_2 was added to the beaker and kept at 60 °C and 80 rpm for 1–3 days. The samples were then passed through Whatman GF/C filters, the filters were placed in glass Petri dishes and stored at room temperature for analysis [36].

Instrumental analysis

Visual analysis

The entire surface of the filters was examined under a stereomicroscope (Leica S6D). Any particles suspected of being microplastics were transferred to a clean filter using a trolley and the image was recorded (Leica EC3, Leica Microsystems, Switzerland). Additionally, the National Institutes of Health (NIH)'s ImageJ application was used to analyze the recorded photos (Version 1.53a; Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997–2014). Following the determination of particle size, the particles were further classified according to their morphologies as fragment, film, and fiber [41].

Polymer structure

An attenuated total reflection Fourier transform infrared spectrometer (PerkinElmer, Spectrum 100) was used to confirm the identity of all particles suspected of being MP. Particles were placed on an ATR crystal, and spectra were measured in the 4000–650 cm-1 range with a resolution of 4 cm-1 (32 scans) (Fig. 3). The obtained spectra were compared with reference polymer spectra, and only particles with > 70% match were verified as MPs [12].

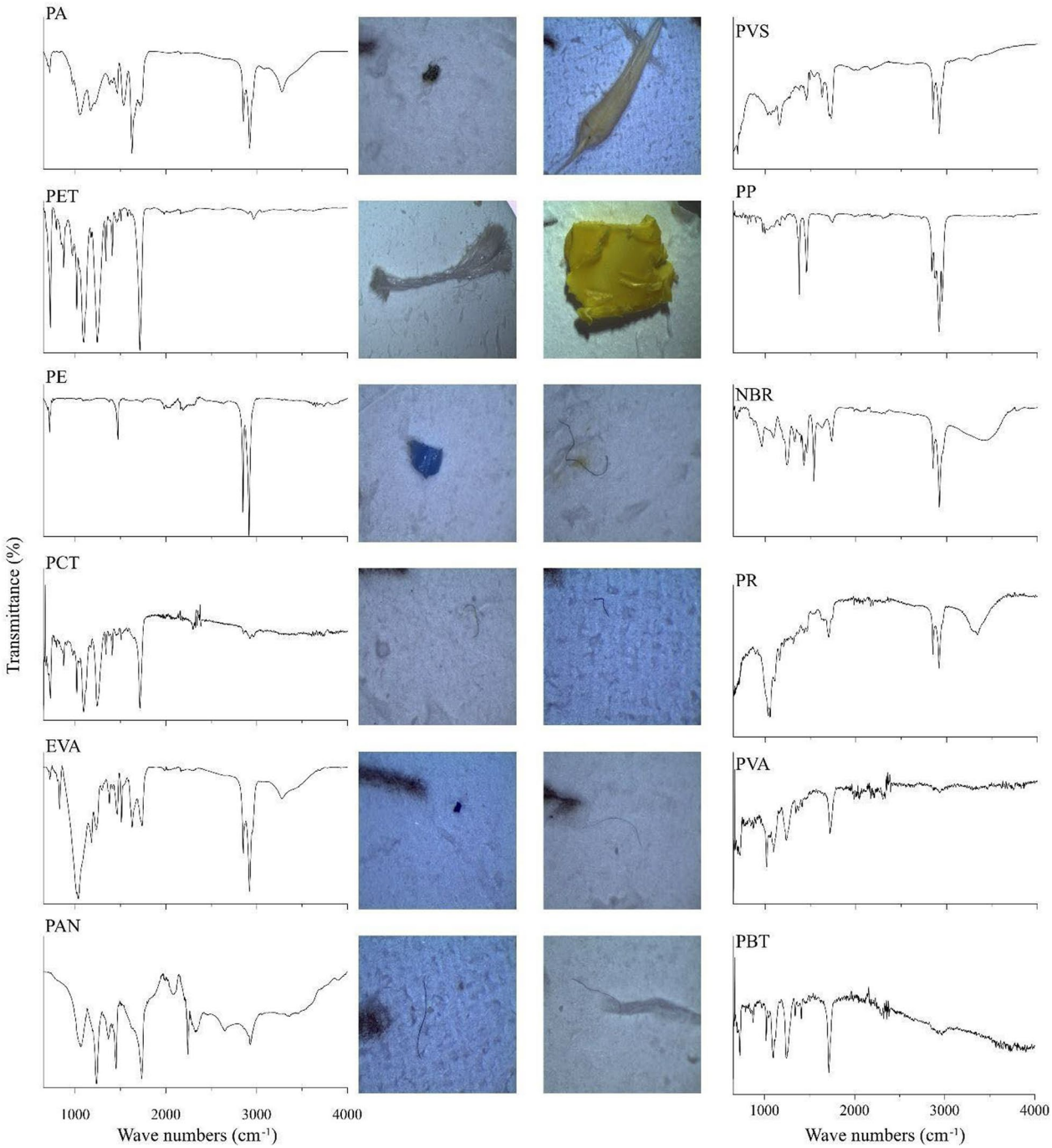


Fig. 3 FTIR spectra and stereomicroscope images of microplastics detected in the samples

Quality assurance and control

To prevent the presence of microparticles throughout the entire experiment, strict preventive measures were enforced in the lab. These included limiting airflow through the ventilation system, managing access to and from the lab, and thoroughly cleaning all work surfaces. Experiments were conducted using cotton aprons and nitrile gloves. Negative blank tests were also performed. Empty beakers were utilized with GIT and sediment samples, replicating the same process as actual samples. Microscopic examination of a clean Petri dish involved adding filtered water to check for airborne microplastic contamination. Any microplastics detected from negative blanks were deducted from the total count. To determine the recovery rate for GIT digestion and sediment density separation, three sets of positive control samples ($N=3$) were employed. This involved placing pre-prepared PE, PP, and PET particles (150–212 µm) into the beakers. Furthermore, 0.5 g of muscle tissue from a frog's hind leg was used, and MPs were extracted as described by Tatlı et al. [36]. H_2O_2 was subsequently added to the muscle tissue, and the procedure followed the same methodology used for the regular samples. For the sediment, density separation was first performed with 500 mL of $ZnCl_2$, using approximately 100 g of the sampled sediment. The particles that collected in the supernatant were then extracted. The $ZnCl_2$ solution was removed, and the sediment underwent digestion at 550 °C for 24 h to eliminate organic matter and any potential MPs. Next, the pre-prepared PET, PE, and PP particles were introduced to the sediment and thoroughly mixed. Density separation and $ZnCl_2$ filtration were then repeated in the same way as for the collected samples. For both tissue and sediment, particles captured on the filter were counted using a stereomicroscope, and recovery was calculated by dividing the number of particles added by the number extracted. The recovery rates were found to be 96.7 ± 2.9%, 93.3 ± 2.9%, and 98.3 ± 2.9% for PE, PP, and PET, respectively.

Data analysis

The Shapiro–Wilk test was employed to assess the normality of the data of length, body weight, and MP abundance detected in GIT, tadpoles, water, sediment samples, and MP lengths. The homogeneity of variances in the adult frogs was then evaluated using Levene's test. A one-way analysis of variance (ANOVA) was used to test for variations in SVL and weight in adult frogs at the sampling locations. The Kruskal–Wallis test (K-W) was employed to analyze the spatial variation of MP abundance and the variation of MP sizes concerning MP shapes. Spearman's correlation was employed to investigate the correlation between the SVL and weight of the frogs and the abundance of MPs in their GITs. PERMANOVA was employed to ascertain the distinctions between MP polymer types and shapes at varying sampling locations. The data were analyzed using JMP Statistical Software. All identified MPs were recorded according to length, polymer type, shape, and color. MP abundance was quantified as the total number present in each sampling location and as the number of MPs per individual.

Results

Blank samples had an average of 0.35 ± 0.27 MP filter^{-1}. By visual inspection, the average number of MPs in the blanks after filtration was less than one. The number of MPs detected in the blanks was subtracted from the total number of MPs in the batch. The results of the recovery test for tissue and sediment are as follows. For tissue, PET, PE, and PP were 93.3%, 95%, and 96.7%, respectively, while for sediment, they were 95%, 91.7%, and 98.3%, respectively.

Microplastics in sediment and water

The amount of MPs in the sampled sediments was distributed between 0 and 0.03 ± 0.01 MP g^{-1}. In 7 out of 11 stations, no MP was detected. Of the MP detected, 75% were fibers, and the remaining 25% were fragments (Table 1). Regarding polymer types, 50% were PCT, and the remainder were PE. While 50% of the MPs detected were blue, 25% were black, and the remaining 25% were orange (Table 1). While the MP lengths were distributed between 133 and 563 µm, the average was calculated to be 379 ± 179 µm. Similarly, MP was detected in water samples from 4 stations, while MP was not in 7 (Table 1). The abundance of MP in the water samples varied between 0 and 7.20 ± 1.06 MP L^{-1}. While 75% of the detected MPs were fibers, 25% were fragments. Polymer types were ethylene–vinyl acetate (EVA), Polyamide (PA), polycyclohexylenedimethylene terephthalate (PCT), and Polyethylene (PE), and all MPs were blue for water samples. MP lengths were found to vary between 151 and 235 µm with an average of 194 ± 37 µm.

Microplastics in tadpoles

The number and weight of tadpoles collected from each station are given in Table 2. Tadpoles were sampled at seven stations. No tadpoles were found at the other 4 stations. The frequency of MP detected in tadpoles is also given in Table 2. Only one MP was detected at Istanbul and Antalya stations and calculated as 0.08 and 0.20 MP ind^{-1}, respectively. The MP detected in the tadpoles sampled in İstanbul were fiber-shaped, PET, and blue, while in Antalya, they were fragment-shaped, polyvinyl stearate (PVS), and white. The lengths were 174 µm for fiber and 1056 µm for fragment.

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

ID	Sampling location	MP shape	MP type	Color	Length (µm)	MP g^{-1} or L^{-1}
Sediment						
1	İstanbul	–	–	–	–	–
2	Bursa	Fiber	PCT	Blue	563	0.03 ± 0.01
3	Manisa	–	–	–	–	–
4	Antalya	Fiber	PCT	Black	407	0.03 ± 0.01
5	Burdur	–	–	–	–	–
6	Isparta	–	–	–	–	–
7	Adana	–	–	–	–	–
8	Kayseri	–	–	–	–	–
9	Şanlıurfa	Fragment	PE	Orange	413	0.02 ± 0.00
10	Erzincan	Fiber	PE	Blue	133	0.03 ± 0.01
11	Samsun	–	–	–	–	–
Water						
1	İstanbul	–	–	–	–	–
2	Bursa	Fiber	PA	Blue	235	7.20 ± 1.06
3	Manisa	–	–	–	–	–
4	Antalya	–	–	–	–	–
5	Burdur	–	–	–	–	–
6	Isparta	–	–	–	–	–
7	Adana	–	–	–	–	–
8	Kayseri	Fiber	EVA	Blue	177	0.27 ± 0.12
9	Şanlıurfa	–	–	–	–	–
10	Erzincan	Fiber	PCT	Blue	216	0.07 ± 0.12
11	Samsun	Fragment	PE	Blue	151	0.53 ± 0.31

Table 2 Microplastic characterization in tadpole samples collected from Türkiye

ID	Sampling location	*N*	Total weight (g)	Total MP	MP shape	MP type	Color	Length (µm)	MP ind^{-1}	MP g^{-1}
1	İstanbul	12	22	1	Fiber	PET	Blue	174	0.08	0.05
2	Bursa	–	–	–	–	–	–			–
3	Manisa	–	–	–	–	–	–			–
4	Antalya	5	0.5	1	Fragment	PVS	White	1056	0.20	2.00
5	Burdur	45	1.5	–	–	–	–			–
6	Isparta	14	4.5	–	–	–	–			–
7	Adana	–	–	–	–	–	–			–
8	Kayseri	44	1	–	–	–	–			–
9	Şanlıurfa	24	2	–	–	–	–			–
10	Erzincan	13	2.5	–	–	–	–			–
11	Samsun	–	–	–	–	–	–			–
Overall		157	34	2					0.01	0.06

MPs in adult frogs

A total of 100 adult frogs were sampled, with an average length of 65.10 ± 18.42 mm and an average weight of 43.48 ± 41.04 g (Table 3). Despite the existence of discrepancies in the length and weight of adult frogs between sampling sites, these discrepancies were not statistically significant (ANOVA, $p > 0.05$). Except for Station 7 (Adana), the presence of MP was confirmed in at least one of the gastrointestinal tracts of the frogs sampled from the remaining sites (Table 3 and Fig. 4).

Table 3 Characteristics of the sampled frogs and the microplastics detected in these frogs collected from Türkiye

ID	Location	Species	*N*	SVL (mm)	Weight (g)	[a]MP (ind.)	[b]FO%	[c]Total MP	[d]MP ind^{-1}
1	İstanbul	*P.ridibundus*	10	57.07 ± 11.74	23.86 ± 13.03	6	60	42	4.20
2	Bursa		17	48.04 ± 14.59	18.70 ± 16.57	7	41	25	1.47
3	Manisa	*P.bedriagae*	10	77.07 ± 16.43	84.58 ± 5.51	7	70	33	3.30
4	Antalya		5	74.27 ± 9.67	48.82 ± 18.00	3	60	8	1.60
5	Burdur	*P. caralitanus*	5	59.26 ± 4.28	23.95 ± 4.65	4	80	7	1.40
6	Isparta		11	96.61 ± 17.21	128.42 ± 54.84	6	55	22	2.00
7	Adana	*P.ridibundus*	7	62.51 ± 8.69	26.85 ± 10.87	0	0	0	0.00
8	Kayseri		10	57.81 ± 5.58	20.19 ± 5.89	3	30	9	0.90
9	Şanlıurfa		5	57.22 ± 9.67	24.48 ± 10.04	1	20	1	0.20
10	Erzincan		10	69.19 ± 10.11	34.43 ± 20.01	3	30	3	0.30
11	Samsun		10	62.82 ± 12.64	31.29 ± 16.16	4	40	13	1.30
Overall			100	65.10 ± 18.42	43.18 ± 41.04	44	44	163	1.52

Total length (mean ± SD), weight (mean ± SD), the total number of frogs analyzed (*N*), ind: individual

[a] Number of frog that ingested microplastics (MPs)

[b] Frequency of occurrence (FO)

[c] Total number of MP found in gastrointestinal tracts (GIT)

[d] Average MP individual^{-1}

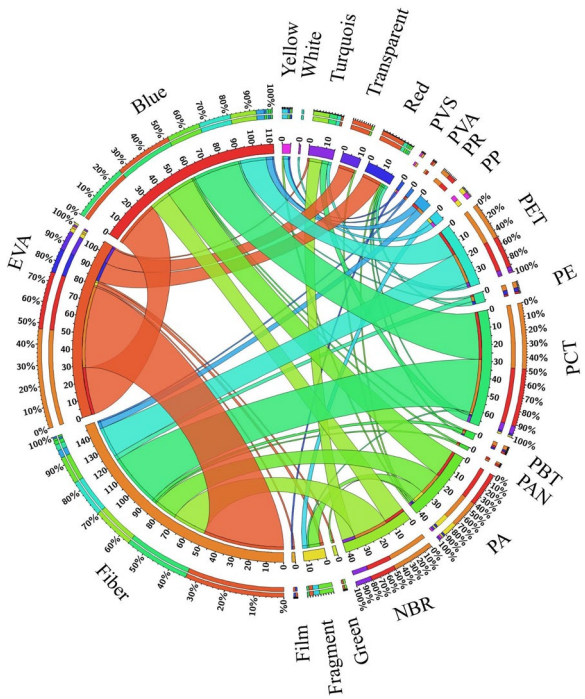


Fig. 4 Microplastic characterization in adult frogs collected from Türkiye

At the species level, the mean MP per individual for *P. ridibundus* was 1.33 ± 1.43, while the mean MP per individual for *P. bedriagae* and *P. caralitanus* was 2.8 ± 1.20 and 1.81 ± 0.42, respectively.

No significant difference was found between the species in terms of MP per individual (K-W test, $p > 0.05$).

Overall, MP was identified in 44 out of the 100 sampled frogs. The distribution of MP in the GITs of adult frogs ranged from 0 to 4.20 MP ind.$^{-1}$, and this distribution was not statistically significant (K-W test, $p > 0.05$) (Fig. 5a). Furthermore, no significant correlation was observed between MP accumulation in the GITs and the snout-vent length (SVL) or weight parameters (Spearman correlation, $p > 0.05$).

A total of 163 MPs were isolated from adult individuals and subjected to characterization (Fig. 4). It was established that MPs exhibited three distinct morphological shapes: 90.8% fiber, 8% fragment, and 1.2% film (Fig. 5a). The distribution of MPs in terms of shape was consistent across the stations (Fig. 5b; PERMANOVA, $p > 0.05$). The detected MPs were classified into 12 distinct groups based on polymer types (Fig. 3). The polymer species were ranked in order of abundance as follows: EVA (32.5%), PCT (20.8%), PA (12.9), nitrile butadiene rubber (NBR, 12.9%), and PET (11.7%). The remaining polymers were present in lower quantities, with Polyamide resin (PR, 2.5%), PE (1.8%), PP (1.8%), poly 1,4-butylene terephthalate (PBT, 1.2%), polyacrylonitrile (PAN, 0.6%), polyvinyl alcohol (PVA, 0.6%), and polyvinyl stearate (PVS, 0.6%) representing the most abundant. The distribution of polymer species was also found to be similar at the stations (Fig. 5b; PERMANOVA, $p > 0.05$). The detected microplastics were classified into seven categories based on their color (Fig. 5c). The abundance of each category is as follows: blue (68.7%), red (10.4%), turquoise (9.2%),

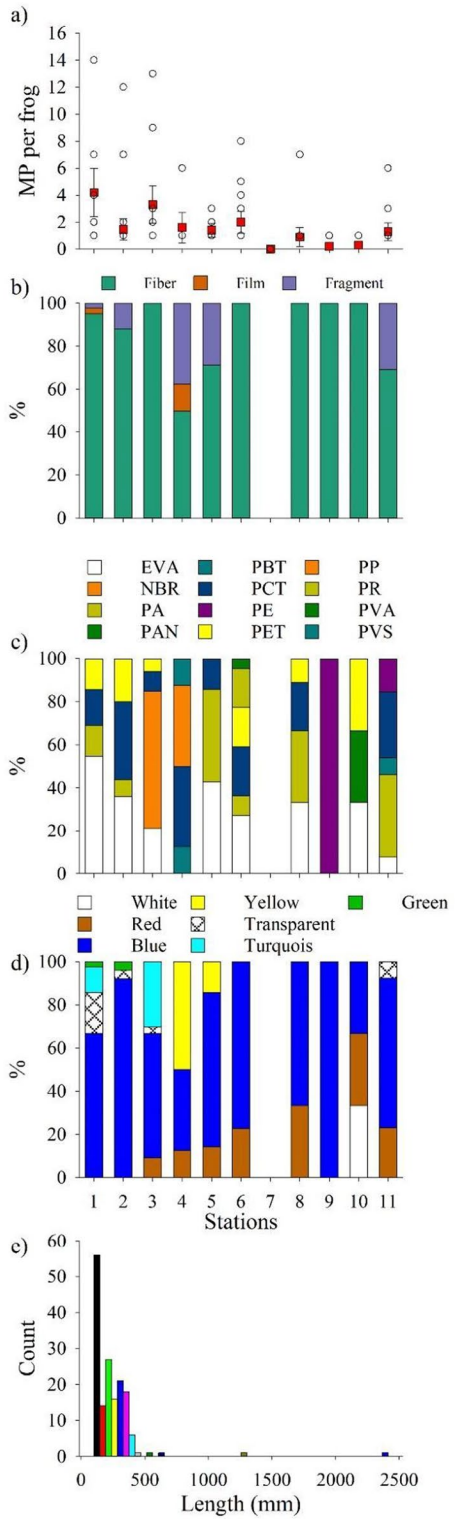


Fig. 5 Microplastic abundance and characteristics in adult frogs sampled from different regions of Türkiye

transparent (6.8%), yellow (3.1%), green (1.2%), and white (0.6%). It was observed that the distribution of colors was similar between the stations, with no significant difference in shape or polymer types (Fig. 5d; PERMANOVA, $p > 0.05$). Figure 5e illustrates the size distribution of the detected MPs. The length of the detected MPs exhibited a range of 100 to 2411.5 µm, with an average of 242.30 ± 215.14 µm. Of the MPs, 161 were determined to be smaller than 500 µm. The most prevalent size group was observed to be between 100 and 200 µm, comprising 76 MPs (Fig. 5e).

Discussion

Eleven distinct locations of Türkiye (Fig. 1) were sampled for their freshwater habitats (lakes, ponds, ditches, etc.), and their MP abundance and characterization were assessed using water, sediment, adult frog, and tadpole samples.

MPs in small water bodies and sediments

The present study revealed the presence of low concentrations of MP in water samples obtained from small water bodies situated in disparate geographical regions. Notably, no MP was detected in some of the sampled stations. The highest values in the water samples were detected at Bursa station (station 2). We observed that the anthropogenic impact during sampling at this station was intense (Table S1). These were the presence of people fishing nearby and the presence of empty plastic bottles and pieces of plastic on the lakeshore. There was also a factory in the immediate vicinity. In general, however, MP was not detected at 64% of the sampling stations, and considering the abundance of MP at the stations where it was found, the difference is not significant (except for station 2). When we compared the data obtained in the study with the literature, we found that the majority of studies carried out in inland waters related to rivers and lakes, and we found a limited number of studies carried out in small water bodies. Looking at other freshwater studies conducted in Türkiye, similarities with our values were observed. Atici et al. [42] found that the MP in the surface water of Lake Van, the largest lake in Türkiye, ranged from 2.35 to 5.09. Another study found 5.25 particles m^{-3} in the water of the Sereyyabey Dam [43]. Another freshwater study was carried out by Gündoğdu et al. [44] in two different rivers, and it was observed that 0.01 MP m^{-3} at station P-4 increased to 28.21 MP m^{-3}. However, when compared with studies conducted in different parts of the world, it can be seen that the microplastic abundance reported for Türkiye is lower than in other countries. For example, Hu et al. [45] found that it varied from 0.48 to 21.52 items L^{-1} in 25 different small water body samples in the Yangtze River Delta, China.

Again, in China (Hangzhou Great Bay Area) and small water body samples, it was reported to be 4.70 ± 2.30 items/L [46]. In addition, as in most of the stations in our study, lake waters were also reported where no MP was detected [33].

In sediment samples, as in water samples, MP was not detected at 7 stations. There was no statistical difference between the stations where MP was detected ($p > 0.05$, Table 1). In general, the stations were located away from population and anthropogenic influences. For this reason, the values obtained were generally lower than those reported in the literature. For example, more MPs were found in sediment samples from Kartalkaya Dam Lake (122 MP kg^{-1}) and Çıldır Lake (83 MP kg^{-1}) [47]. We observed that the dimensions, morphology, and prevalence of these polymers varied according to the field site and sample type, including surface water or sediment. Minor aquatic systems are among the most under-researched environments concerning MPs, despite their essential ecological functions in sustaining biogeochemical cycles and freshwater biodiversity globally [48]. Although the prevalence of MPs in tadpoles and sediments was generally low, site-specific data reveal significant insights into anthropogenic impacts. For instance, the highest MP concentrations were observed in areas like Bursa, characterized by proximity to industrial activity and visible plastic debris. Such findings emphasize that anthropogenic factors, such as improper waste management and urbanization, play a critical role in MP prevalence. However, other factors, including local hydrodynamic conditions and natural sedimentation processes, might also influence MP distribution. Further studies could integrate environmental variables to provide a comprehensive explanation. Consequently, forthcoming research should emphasize the comprehension of the accumulation and possible impacts of plastic particles on the creatures residing in these environments [45].

MPs in tadpoles and adult frogs

Our findings confirmed the prevalence of MPs in tadpoles and adult frogs across different sample sites, indicating that amphibians in freshwater habitats are constantly at risk. Just like in earlier research on MP contamination in frogs [12, 28, 36]. The majority of the MPs found were fiber-shaped, while a minor percentage were fragments and films. Amphibians, especially those in the aquatic stage, depend on filter-feeding and suction processes. The size and shape of microplastics, especially fibers, could make them easier to swallow [12, 49]. Ingesting these fibers can disrupt digestion, absorption of nutrients, and feeding, which can put one's health in danger [4, 50].

The analysis of 157 tadpole samples revealed the presence of only 2 MPs (0.1%). On the other hand, MP was identified in 44% of the 100 adult frogs examined, with a total of 163 microplastics recorded. The greater occurrence of MPs in adult frogs compared to tadpoles may reflect differences in feeding strategies, habitat use, and cumulative exposure over time [33]. Adult frogs, which inhabit both aquatic and terrestrial environments, may encounter MPs through direct ingestion of contaminated prey or indirectly through environmental exposure, such as feeding in areas with high MP concentrations in sediment or water [34, 51]. Tadpoles, however, are confined to aquatic environments, and their exposure to MPs likely comes from sediment particles and periphytic algae [33]. This environmental difference is consistent with the fact that MPs have not been detected in aquatic habitats (sediment and water samples) inhabited by populations (e.g., Adana and Şanlıurfa) where MPs are either absent or only marginally present in tadpoles and adult frogs. The low MP intake in tadpoles compared to adults suggests distinct exposure pathways. Tadpoles' reliance on filter-feeding and periphyton scraping limits MP ingestion to particles adhering to their food sources, whereas adults encounter MPs through a wider array of trophic interactions. These findings align with global observations, such as studies on *Rana temporaria* in Italy [52] and Bangladesh [53], where MP ingestion differed significantly between developmental stages.

The three Anatolian water frog species exhibited varying MP prevalence in their GITs. *P.bedriagae* showed the highest mean MP count per individual, potentially reflecting differences in habitat preferences, feeding behavior, and ecological niches. This species' confinement to agricultural ditches and smaller water bodies might increase exposure to MPs originating from agricultural runoff and local anthropogenic activity [36]. In contrast, *P. ridibundus*, with its broader habitat range, demonstrated a slightly lower prevalence, possibly due to reduced exposure in less contaminated environments. On average, 1.52 MPs were found per adult specimen. The findings of this study indicate that the concentration of MPs in the GITs of Anatolian water frogs is higher than the value of 0.46 MPs per individual reported in a previous study [36]. An additional investigation in a large delta in Bangladesh revealed the presence of microplastics (5–18 MPs per individual) in the GITs of 90% of the nine amphibian species sampled [53]. Furthermore, the average count of MPs in tadpoles from five distinct *Rana temporaria* species was recorded as 0.35 MP per individual in southwestern Poland [54] and 0.33 MP per individual in Lower Lake Balma, northern Italy [52], which were higher than the values obtained in our study (0.01 MP/individual).

Researchers have been interested in the subject of whether MP abundance correlates with length/weight. Although most research on amphibian species has found no link between MP abundance and length/weight data [28, 36, 53, 54], one study [12] found a positive and statistically significant relationship. No correlation between MP abundance and length/weight following the overall tendency was noted in the studied species that were investigated.

The dimensions of microplastics are of great consequence, as diminutive particles have the potential to collect at lower levels of the food chain [55] and influence the residence time within organisms [56, 57]. Studies have confirmed that the effect of MPs on aquatic life is more dependent on their size rather than the type of polymer (Andrady 2011 [58],). The most prevalent size group in adult frogs was identified as 100–200 µm, comprising 76 particles, which aligns with the range observed in prior studies (100–150 µm) [36, 52]. However, a study conducted in Bangladesh revealed that the most prevalent length range of MPs was 1.000–5.000 µm, representing 36% of the total MPs [53]. A subsequent study identified specific histopathological alterations induced by MPs in tadpoles [59]. In the present study, very few MPs were detected in tadpoles, limiting our evaluation in terms of MP size. The presence of MPs smaller than 200 µm in more than half of our samples implies that these particles could have been introduced into the food webs at a lower trophic level.

Adult frogs and tadpoles were found to contain 12 different polymers, including EVA, NBR, PA, PCT, PE, PAN, PBT, PET, PP, PR, PVA, and PVS. When all locations were considered together, the proportion of EVA polymer types was found to be the highest (32%), followed by PCT (21%) and PA (13%). These three materials collectively constituted 66% of the total microplastics, while the remaining nine material types accounted for only 34%. Türkiye is expected to produce 10.9 million tonnes of plastic in 2023, with EVA polymers contributing approximately 2% (200 thousand tonnes) to this volume (Turkish Plastic Industry Foundation, PAGEV, 2023). EVA's toxicity to organisms is classified as moderate by biological and physical evaluations [60]. Research on adult frogs in Türkiye has shown similar outcomes, with PET, EVA, and PCT polymers making up 95% of the total MPs found [36]. Conversely, microplastics identified in adult common frogs in the Italian Alps were mainly composed of polyamide (PA) [33]. Studies on tadpoles have also reported frequent presence of PA and polyester (PES) MPs [46, 54]. The presence of microplastics in aquatic ecosystems is associated with several factors, incorporating the use of plastic items, the efficacy of waste management practices, and the aggregation of plastic debris conveyed by wind and precipitation [61].

The color of the plastic makes it easier for researchers to identify possible origins of MPs [62, 63]. In addition to blue and white MPs in tadpoles, red, yellow, teal, turquoise, green, and transparent MPs were detected in the GITs of adult frogs. Similar results were found in previous studies of amphibian tadpoles and adult frogs [36, 52, 54]. Although marine organisms often ingest blue microplastics due to the resemblance of these plastics to their natural prey [64, 65], this explanation may not apply to amphibians. For example, plastic bags, fabrics, or fishing nets of different colors may account for the diversity of MP colors found in amphibians [53].

The ecological and physiological consequences of MP ingestion for amphibians remain speculative but warrant further exploration. Potential impacts include physical blockage of the digestive tract, reduced nutrient absorption, and exposure to toxic chemicals adsorbed onto plastic particles [10]. Long-term effects could manifest as reduced reproductive capacity and population declines [20]. Additionally, the presence of MPs in amphibians indicates potential trophic transfer, posing risks to higher trophic levels and ecosystem stability [51]. Experimental studies are needed to elucidate these effects comprehensively [66]. This study underscores the urgent need for targeted conservation efforts to mitigate MP pollution in freshwater ecosystems. Specific actions include improving waste management systems, reducing plastic usage in agriculture, and restoring critical amphibian habitats. Policy interventions should prioritize regions with high MP prevalence and promote public awareness to address the broader issue of plastic pollution.

Future research should focus on longitudinal studies and experimental approaches to unravel the complex interactions between MPs, amphibians, and their habitats.

Conclusions

The prevalence and features of microplastic pollution in Türkiye's freshwater ecosystems and amphibian species are brought to light in this study. The low concentrations of MPs in water and sediment samples, except for more populated regions, point to the possibility of regional contamination. The different eating habits and cumulative exposure concerns are highlighted by the increased frequency and diversity of MPs in adult frogs compared to tadpoles. Common environmental toxins are reflected by fiber-shaped MPs and other types of polymers, such as EVA and PCT. The findings highlight the environmentally dangerous situation for amphibians and the possibility of MPs bioaccumulating in food chains. The impacts of MP exposure on amphibian health and development

over the long term, together with its wider consequences for biodiversity and ecological stability, should be the subject of future studies. Protecting amphibian habitats and decreasing plastic pollution should be the top conservation priorities.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-025-01065-1.

Supplementary Material 1.

Author contributions

A.A. and K.G. wrote the main manuscript text and H.T. prepared Fig. 2. K.G. prepared Figs. 1, 3, 4, 5 and Tables 1, 2, 3. 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

The local ethics council at Recep Tayyip Erdogan University granted permission to conduct the study, and all specimens used in it were handled humanely and ethically (decision number: RTEÜ.2021/15).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests. matrilas

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