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PRIMARY RESEARCH PAPER

Changes in microplastic-associated bacterial communities along a salinity gradient in Central Anatolian lakes of Türkiye

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Abstract Lakes face anthropogenic stressors such as climate change, salinization, and eutrophication, and microplastics (MPs) now emerge as a new concern since they act as vectors for pathogenic bacteria, giving rise to concern about environmental health. This study explores cultivable viable bacterial (CVB) biofilm communities on MPs along a salinity gradient (0.25 to 192.69‰) in 11 lakes in Türkiye. The MPs consisted mainly of fibers and 50-µm to 5-mm-sized fragments. The dominant cultivable bacterial phyla in both MP-associated biofilms and lake water were *Proteobacteria* and *Firmicutes*. On MPs, *Pseudomonadaceae* was the dominant family (94.7%), followed by *Enterobacteriaceae* (1.2%). Various halolerant pathogens occurred as well, e.g., *Proteus* spp. strains on MP surfaces in Lake Bolluk (95.7‰) and *Alcaligenes* spp. in Lake Tuz, the two lakes with the highest salinity, while the halophilic *Shewanella putrefaciens* was prevalent in highly alkaline Lake Salda. *Staphylococcus*, *Commomonas*, and *Enterobacter* were

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found in all lakes regardless of salinity level, and CVB strains were more common on MPs surfaces than in lake water. Our findings suggest that MPs promote the development, dispersion, and bioavailability of bacteria, including pathogens, thus increasing ecological and health-related impacts in lakes under different salinity conditions.

Keywords Plastisphere · Freshwater salinization · Vector role · Bacterial biofilm · Microplastics · Pathogens

Introduction

Plastics have become one of the most important human-made industrial materials (PlasticsEurope, 2021) and play a crucial role in our daily lives. This was highlighted during the COVID-19 pandemic when plastics became important for medical safety measures, as testing tools, and for various other applications (Parashar & Hait, 2021). The worldwide production of nonrecyclable plastics has surged from 2 million metric tons in 1950 to approximately 430 million metric tons in 2023 (United Nations Development Programme, 2024), encompassing various materials such as thermoplastics, polyurethanes, thermosets, elastomers, adhesives, coatings, sealants, and PP fibers (PlasticsEurope, 2021). Inadequate waste management and widespread littering have resulted in the occurrence of a novel long-term persistent pollutant with profound impacts on aquatic environments—microplastics (Mol & Caldas, 2020). The breakdown of plastics, producing microplastics (<5 mm), is influenced by various factors, including physical (solar UV radiation, currents, etc.) and biological factors (e.g., bacterial consumption and degradation). The heterogeneous nature of MPs, encompassing various types, colors, and sizes, introduces the risk of direct ingestion or ingestion via prey items by various organisms, including fish (Cera et al., 2022), zoobenthos (Hara et al., 2020; Scott et al., 2019), birds, turtles, and mammals (Duncan et al., 2019; Hamilton et al., 2021; Moore et al., 2020). Additionally, the surface properties and chemical structures of MPs make them act as a vector, facilitating the transport of harmful organic chemicals, additives in plastics, and pathogenic organisms, which in combination, through trophic transfer, create toxic effects (Baldwin et al., 2016; Başaran Kankılıç et al., 2023; Ockenden et al., 2021). A considerable number of studies document the presence of MPs in marine environments, but also the presence of MPs in lakes and rivers has been extensively documented in recent years (e.g., Baldwin et al., 2016; Devereux et al., 2023; Eriksen et al., 2013; Free et al., 2014; Mao et al., 2020; Pan et al., 2020). Notwithstanding the emerging evidence of the adverse effects of MPs, knowledge about the transport, and fate of microplastics in inland waters remains limited, particularly in less studied regions such as Türkiye (Atici et al., 2022; Çomaklı et al., 2020; Tavşanoğlu et al., 2020).

Plastic debris and MPs have multiple impacts on aquatic ecosystems (Issac & Kandasubramanian, 2021). They provide a suspended surface that can be colonized by microorganisms and support the development of microbial biofilms (Amaral-Zettler et al., 2020; Zettler et al., 2013). Biofilms are complex assemblages of algae, bacteria, protozoans, and fungi embedded in an extracellular matrix that grows on different surfaces. Microbial biofilm developed on plastic debris is referred to as the "plastisphere" (Zettler et al., 2013). MPs are known to host a wide variety of microorganisms, including pathogens and species associated with harmful algal blooms, which can be transported over long distances (Di Pippo et al., 2020; Masó et al., 2003; Reisser et al., 2014; Semcesen & Wells, 2021; Zettler et al., 2013). Research has shown that these microbial communities can be specific to MPs, having distinct patterns compared to those found in the surrounding aquatic environment (Di Pippo et al., 2020; Oberbeckmann et al., 2014).

Biofilm growth is highly influenced by the structure and complexity of the substrates upon which it develops, affecting both biomass accrual and community composition (Hao et al., 2017; Pacheco

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et al., 2022). The physical characteristics of plastics, encompassing roughness, hydrophilicity, and hydrophobicity, play a pivotal role in influencing the growth and composition of plastisphere biofilms. Furthermore, spatial and environmental variations can shape the microbial community composition of biofilm on MPs (Hoellein et al., 2017; Kettner et al., 2019; Oberbeckmann et al., 2014). The development of the biofilm community on the MPs often depends on the abiotic conditions, with salinity being one of the key factors shaping the plastisphere (Song et al., 2022; Zettler et al., 2013). However, our understanding is limited of the dynamics governing biofilms associated with MPs and their ability to effectively disperse these microorganisms in diverse aquatic ecosystems characterized by variations in salinity (Di Pippo et al., 2022; Kettner et al., 2019; Song et al., 2022).

Spatial studies conducted along salinity gradients may help to improve our knowledge about the role of salinity and related physicochemical factors affecting the diversity of microorganisms growing on MPs. Increases in salinity result in higher water density, which promotes persistent suspension of particles in the water column. However, this behavior depends on physical factors such as flow velocity, mixing, and turbidity (Mendrik et al., 2023). Salinity significantly impacts the bacterial composition in aquatic environments, inhibiting the activity of various microorganisms and influencing bacterial proliferation, especially under high-salinity conditions (Dong et al., 2018). While knowledge exists of bacterial alterations on the surface of MPs upon entering marine ecosystems (e.g., Song et al., 2022), our understanding of this topic in lakes along a large salinity gradient is still scarce. The variety and quantity of sampling sites, the regional scale and varying hydrological conditions, and the consideration of different catchment areas still require research to better understand their effects on the prevalence of microplastics and the plastisphere community in lakes (Baldwin et al., 2016).

The primary aim of this study was to evaluate the bacterial biofilm composition associated with MPs across a gradient from freshwater to highly saline lakes. Conventional microbiological methods acknowledged as the best standard for validating the viability and physiology of the isolated strains (Azeredo et al., 2017) were employed to achieve this goal. We hypothesized that salinity plays a pivotal role for the composition of bacterial plastisphere communities in lakes. Our study advances the understanding of the role on MPs in the composition of the bacterial biofilm and the potential differences caused by lake water along a salinity gradient. Our findings contribute to the knowledge of the impacts of salinity and land use on the distribution, sources, and biofilm community of MPs as well as the role of MPs for the presence and distribution of bacteria, including those that are potential pathogens in inland aquatic ecosystems.

Materials and methods

Study area

In July 2021, we conducted a lake expedition in the Konya Closed Basin (KCB) and Burdur Closed Basin (BCB) in Türkiye (Çolak et al., 2022; Yılmaz et al., 2021). Here, a total of 11 lakes, covering a wide salinity gradient ranging from 0.25 to 192.69‰, were sampled for microplastics (Fig. 1; Table 1). KCB, the largest closed basin of Türkiye, covers an area of nearly 50,000 km^2 or approximately 7% of the country's area. This region is home to a population of 3.2 million people and serves as a vital hub for extensive agricultural activities (Yılmaz et al., 2021) whose success relies heavily on both surface water and groundwater resources. Consequently, numerous streams have been subjected to regulation and channelization for dam construction to meet the irrigation demands (Yılmaz et al. 2021). Agricultural activities in KCB have played a significant role in the observed changes in salinity and water surface area over the last 40 years (Yılmaz et al., 2021), encompassing groundwater abstraction for irrigation along with limited rainfall and high evapotranspiration, further contributing to the process of salinization (Çolak et al., 2022; Yılmaz et al., 2021).

BCB covers an area of 6296 km^2 in the southwest of Türkiye. It is a mountainous region with an average elevation of 1100 m, with deep lakes originating from the Taurus graben formation during the Miocene and Pliocene periods (Verhaert et al., 2006). The surface water in the BCB is primarily sourced from subbasins like Acıgöl, Akgöl, Salda, and Burdur, located in the northeastern part of the BCB. Due to

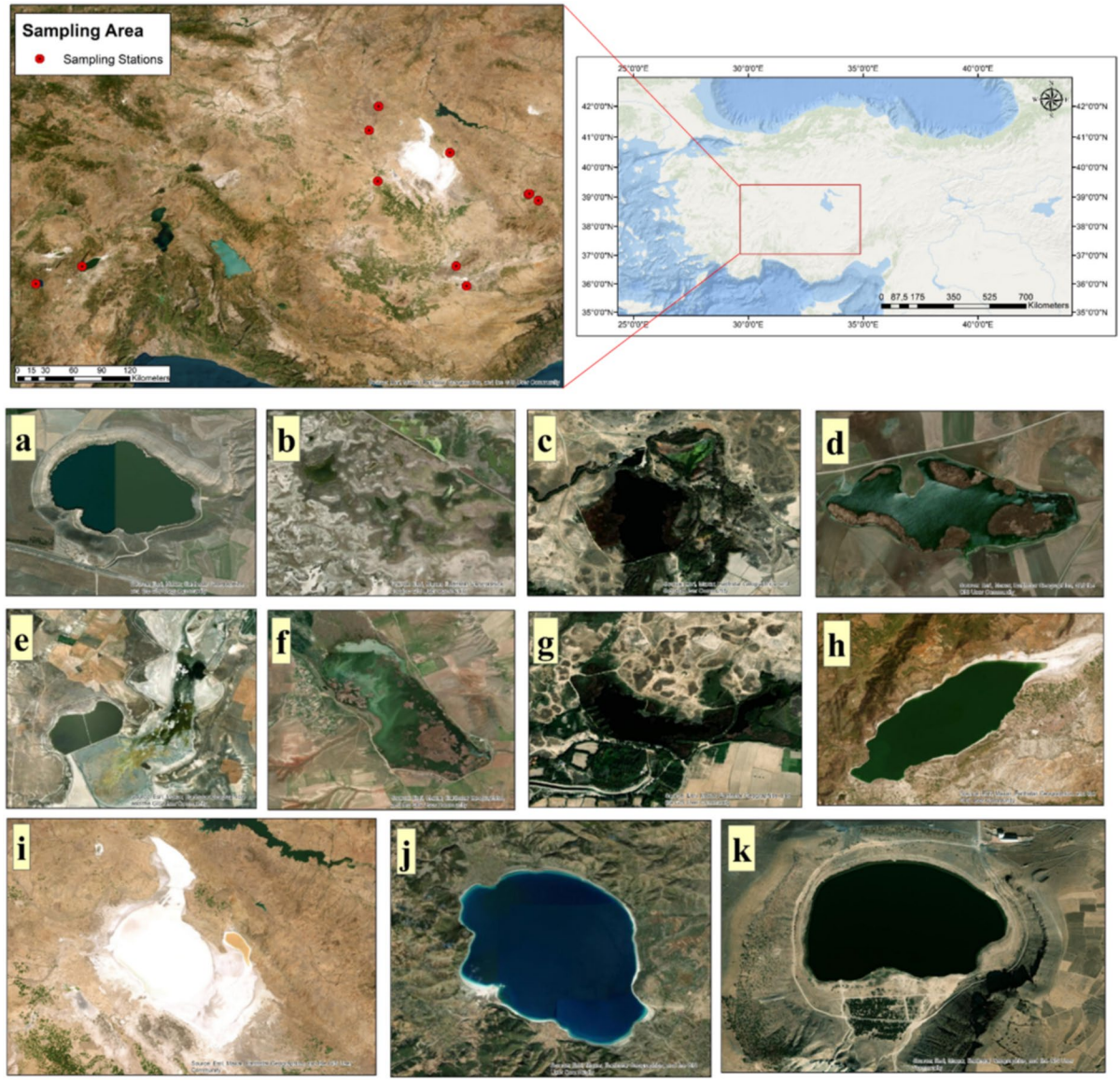


Fig. 1 Study sites: **a** Lake Acıgöl, **b** Lake Akgöl, **c** Lake Kaya, **d** Lake Uyuz, **e** Lake Bolluk, **f** Lake Gökgöl, **g** Lake Baki, **h** Lake Burdur, **i** Lake Tuz, **j** Lake Salda, **k** Lake Narlı

its karstic geology, precipitation is the main contribution to the formation of groundwater in this area. According to Coordination of Information on the Environment Land Cover (CORINE), the land-use pattern is predominantly nonirrigated agricultural land (Fig. 1). However, crop-farming irrigation in the BCB has increased substantially since 2004, resulting in a major increase in lake water abstraction from the lakes, their inflows, and groundwater sources to meet the growing irrigation demands. This has led to a notable increase in water loss in the basin, resulting in a significant reduction in the size of shallow lakes over the past four decades (Çolak et al., 2022).

Sampling

Physicochemical variables—temperature, salinity, conductivity (EC), dissolved oxygen (DO) concentration, and pH—were measured using a YSI 650 MDS at each sampling site. To investigate the bacterial

Table 1 General characteristics of the sampled lakes

Lakes	Origin	Surface area (km^2)	Max. Depth (m)	Temp. (°C)	Salinity (‰)	EC (mS/cm)	pH
Bakıgöl	Aluvial	0.23	4.5	22.57	0.25	0.65	6.61
Kayagöl	Aluvial	0.13	2.5	25.01	0.26	0.67	7.33
Salda	Karstic	44.51	184	23.85	0.91	2.04	9.35
Gökgöl	Aluvial	0.71	1.4	22.01	1.00	1.61	8.56
Uyuz	Aluvial	0.31	1.5	18.61	1.64	2.77	9.58
Narlı	Volcanic	0.50	19.5	24.66	2.43	4.18	8.33
Akgöl	Aluvial	5.47	1.5	30.80	2.88	4.89	9.65
Burdur	Tectonic	203.11	110	27.23	22.43	28.61	9.07
Acıgöl	Volcanic	1.47	85	26.30	68.83	88.93	8.06
Bolluk	Tectonic	11.18	1.5	26.03	95.74	111.0	8.77
Tuz	Aluvial	1939.46	0.5	29.08	192.69	200.0	7.4

Temp temperature, *EC* electrical conductivity

biofilm on MPs in each lake, depth-integrated water column samples were collected using a 5-l Ruttner sampler (KC Denmark). From the pooled samples, 40 l was subsequently filtered through a 300-µm mesh and then transferred to a sterile 250-ml glass bottle for MPs analysis. Simultaneously, 500-ml water samples were collected in a sterilized glass bottle to determine the ambient bacterial composition in the water column. All samples were stored under dark and cold conditions until laboratory analyses.

Laboratory processes

Biofilm analysis

The MPs found in the water samples were sorted according to type under laminar flow to avoid contamination. After separation, each MP was rinsed with sterile physiological saline solution to remove unattached or loosely attached bacteria and then transferred into 1.5-ml autoclave-sterilized Eppendorf® tubes containing 1-ml sterile physiological saline solution, followed by mechanical vortexing for two minutes to detach bacterial cells.

Water samples collected from each site (500 ml) were filtered under a + vacuum through a sterile cellulose 0.22-µm pore size acetate membrane filter (IsoLab, Germany). Next, the filters were placed in sterile falcon tubes with 10-ml sterile distilled water. After vortexing, both the filter samples and the MPs were serially diluted in sterile physiological saline solution as 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}, and subsamples (0.1 ml) were prepared in sterile physiological saline solution (see details in Tavşanoğlu et al., 2020). All water and MPs samples were cultivated on selective/distinctive, specific chromogenic media (R2A agar with 5% NaCl, 5% sheep blood agar, fecal coliform (mFC) agar, eosin–methylene-blue (EMB) agar, McConkey agar, xylose lysine deoxycholate agar (XLD), and Chromocult Enterobacter Sakazakii agar (all from Merck, Germany), UTIC and E. coli-coliforms chromogenic agar (Condalab, Spain), cetrimide agar (LabM, UK)) at 34 ± 0.5 °C under aerobic and microaerophilic conditions (5% CO_2) for 24–72 h. Conventional microbiological methods, including gram staining, examination of colony pigmentation, morphology, motility, biochemical properties, and other related properties for isolation and identification of bacterial strains (Muñoz-Torres et al. 2023; Tavşanoğlu et al., 2020), were also used in the study. Besides, for fastidious bacterial species such as *Nocardia* sp. and *Actinomyces* sp., the incubation period for blood agar media was extended to 15 days. The number of grown colonies of the bacterial species was counted and calculated as colony-forming unit (CFU/ml). Additionally, colonies that could not be identified using conventional methods were isolated as single colonies by passaging and subjected to identification applying a device for automated bacterial typing—MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) (VITEK MS v3.0, BioMérieux, Marcy; l'Etoile, France) (Ashfaq et al., 2022). Furthermore, unidentified strains were characterized at molecular level using the Sanger

DNA sequencing method. DNA isolation was performed using GeneJet Genomic DNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's instructions for gram-positive and gram-negative bacteria. The whole 16S rRNA gene, including V1-V9 regions, was PCR-amplified from total DNA using the bacterial primer pair: universal 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Janda & Abbott, 2007; Lane, 1991) based on TEMPase Hot Start DNA Polymerase (Ampliqon, Denmark). The PCR product was purified using QIA quick PCR Purification Kit (Qiagen) following the manufacturer's instruction, and the products were sequenced using an ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Perkin–Elmer Applied Biosystem). PCR was performed in a 50-µl reaction mixture containing 200 µM dNTPs, 25 mM $MgCl_2$, 20 pmol of both forward and reverse primers, 1U of Hot Start Taq DNA polymerase, 10X ammonium buffer, and 100 ng genomic DNA. PCR cycling conditions were as follows: initial denaturation at 95 °C for 15 min, followed by 30 cycles of 94 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s, and a final extension step at 72 °C for 10 min. PCR products were then purified, and bidirectional sequencing was performed using both primers using the Sanger DNA sequencing method by Macrogen (Korea). The obtained sequences were blasted using the NCBI BLAST (https://blast.ncbi.nlm.nih.gov/) 16S ribosomal RNA sequence database (bacteria and archaea) and the Megablast program to identify bacteria strains at genus/species level (Boratayn et al., 2013; McGinnis & Madden, 2004).

Microplastics analysis

After the selection of MPs for biofilm analysis, the remaining samples underwent treatment with 30% H_2O_2 to eliminate organic residues and sort MPs. Afterward, the samples were passed through GF/C Whatman filters with a pore size of 1.2 µm using a stainless-steel vacuum filter. The plastic particles were separated using a stereomicroscope (Zeiss, Germany) based on their physical attributes and structures and subsequently categorized as fiber, fragment, film, rubber, and others (GESAMP, 2019). The assessment of MPs particles was carried out utilizing Nile red as a staining agent. To prevent airborne MPs contamination, blank samples were incorporated into the laboratory procedures. The safety measures applied to avoid contamination included thorough cleaning with distilled water of all laboratory equipment such as Petri dishes, tweezers, and beakers and continuous use of a cotton coat and nitrile gloves during laboratory processing.

Data analysis

Prior to the analyses, normality was checked, and when the data did not meet the assumption, we used K-W nonparametric rank test to detect differences between the MPs and the surrounding water. To examine the abundance of bacterial composition in relation to polymer groups, principal component analysis (PCA) was conducted using the "vegan" package in R (R Core Team 2021). Prior to the analyses, Hellinger's transformation was applied to the species data to account for the zero-inflated community matrix with skewed distribution and many zeros (Legendre & Gallagher, 2001). All statistical analyses were performed using R (R Core Team, 2021).

Results

Microplastic abundance and composition

Among the 11 inland lakes spanning a salinity range from 0.25 to 192.69‰, the abundance of detected MPs in each lake exhibited variations independent of salinity (Fig. 2). The mean abundance of MPs was 0.83 ± 0.05 (SE) items/L in the sampled lakes (Fig. 2, Table 2), with the highest MPs concentrations occurring in Lake Burdur (1.75 items/l), Lake Salda (1.45 items/l), and Lake Gökgöl (1.33 items/l). Conversely, lakes Baki and Acıgöl had the lowest MPs concentrations of 0.25 items/l and 0.28 items/l, respectively (Fig. 3). Distinct types of MPs were found in each lake, showing variations also in dominant type (Fig. 3). Out of the 366 detected MPs, fibers emerged as the predominant type, constituting 46.7% (Fig. 3). MPs colors exhibited variation across the sampled lakes, with black (52%), blue (22%), and transparent (13%) being the most prevalent colors (Table 2). MPs sizes ranged from 50 µm to 5 mm, and particles with sizes between 0.5 and 2 mm prevailed in all lakes, constituting an average of 52% (Fig. 4).

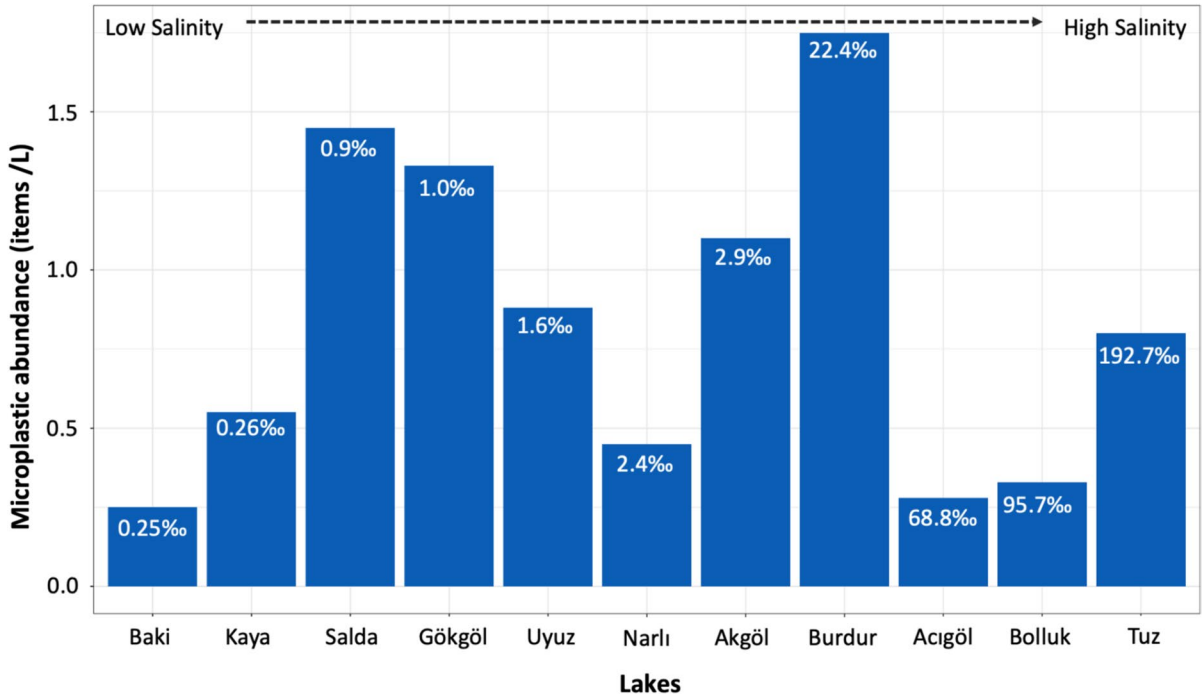


Fig. 2 Microplastic abundance (items/L) in inland lakes along a salinity gradient

Biofilm composition on MPs surfaces and in lake water

The distribution of cultivable bacterial strains exhibited variations among lakes (Fig. 5). Conventional microbiological culture results revealed that Proteobacteria (98.4–83.1%) and Firmicutes (1.6–16.9%) were the most prevalent phyla on the MPs and in the surrounding lake water (Fig. 5). The bacteria *Aeromonas, Enterobacter*, *Pseudomonas*, *Salmonella*, *Staphylococcus*, and *Shewanella* were frequently identified in the lakes along an extensive salinity gradient (0.25–192.69‰) (Fig. 5). We found compositional differences in the bacterial composition between MPs and lake water. The most prominent bacterial families on MPs surfaces were Pseudomonadaceae (94.7%), followed by *Enterobacteriaceae* (1.2%) and *Staphylococcaceae* (1.1%), while the dominant bacteria family in the lake water was *Shewanellaceae* (40.3%), followed by *Comamonadaceae* (25.4%), *Staphylococcaceae* (21.4%), *Enterococcaceae* (6.0%), and Enterobacteriaceae (4.2%). While there were similarities in the number of bacterial strains (CFU/ml) between the surrounding water and MPs surfaces, significant differences emerged regarding the presence of the pathogen *Vibrio* spp., which was notably found in the water of Lake Burdur (Table 3). Furthermore, the presence of the moderately halophilic gram-negative *Salinivibrio* was exclusively detected on the surface of MPs from Lake Burdur. In addition, pathogenic bacteria, including *Aeromonas hydrophyla*, *Comamonas aquatica*, *Enterobacter aerogenes,* and *Staphylococcus aureus,* posing significant health threats, were identified on all MPs surfaces in all lakes. Additionally, *Comamonas aquatica* and *Shewanella putrefaciens* were prevalent in all lake water samples. Generally, the production of cultivable viable bacterial strains was more pronounced on the surface of MPs than in the water (Table 3), while no cultivable viable bacterial strains were found on MPs surfaces in either Lake Kaya or Lake Narlı or in the surrounding water of Lake Gökgöl (Table 3). Despite the absence of a clear pattern between salinity and MPs concentrations, the biofilm composition on MPs exhibited variations along the salinity gradient. According to the PCA analysis, halotolerant *Proteus* spp. strain colonies on the surface of MPs were associated with Lake Bolluk (95.7‰),

Table 2 Abundance, potential sources, and physical characteristic of microplastics found in the study lakes

Lakes	Abundance (Items/L)	Shapes	Size (μm)	Colors	Potential sources
Baki	0.25	Fiber, film, fragment, rubber	440.3–3519.1	Black, blue, transparent	Amateur fishing, visitor activities, transportation (road)
Kaya	0.55	Fiber, film, fragment, rubber	213.1–2769.4	Black, blue, red, transparent, white	Amateur fishing, transportation (road)
Salda	1.45	Fiber, film, fragment	55.7–2749.8	Black, blue, red, transparent, white	Popular tourist destination, visitor activities, swimming
Gökgöl	1.33	Fiber, film, fragment	141.2–3464.5	Black, blue, green, red, transparent, white	Visitor activities, untreated wastewater, agricultural and industrial drainage in the area
Uyuz	0.88	Fiber, fragment, rubber	124.1–4917.9	Black, blue, red, transparent, white	Agricultural use of surrounding land and stock farming
Narlı	0.45	Fiber, Fragment, Rubber	233.3–2020.7	Black, blue, red, transparent	Popular tourist destination, visitor activities, swimming
Akgöl	1.10	Fiber, fragment, rubber	113–4865.8	Black, blue, red, transparent	Visitor activities, untreated wastewater, agricultural and industrial drainage in the area
Burdur	1.75	Fiber, film, fragment	83.3–3332	Black, blue, red, transparent	Visitor activities, popular tourist destination, trip around the lake with own car or public transportation
Acıgöl	0.28	Fiber, film, fragment	50–3689.7	Black, blue, transparent	Popular tourist destination and visitor activities
Bolluk	0.33	Fiber, film, fragment, rubber	407.8–3205.5	Black, blue, green, transparent, white	Untreated wastewater, industrial drainage, stock farming in the area
Tuz	0.80	Fiber, film	118.9–4808.4	Black, blue, green, red, white	Household, agricultural and industrial drainage, visitor activities

and *Alcaligenes* colonies were associated with the lake with the highest salinity, Lake Tuz (Fig. 6). A high bacterial unit (CFU/ml) of *Shewanella putrefaciens,* commonly known as halophilic bacteria, was recorded at several salinity levels (Table 3), including Lake Salda, which is characterized by high alkalinity (Fig. 5). Simultaneously, the presence of widely distributed bacterial strains, including *Staphylococcus*, *Comamonas*, and *Enterobacter*, was consistently observed in all the sampling lakes regardless of salinity level (Table 3). The PCA analysis of cultivable viable bacteria in the water samples revealed a similar distribution on MPs surfaces (Fig. 6b). For example, halophilic bacteria such as *Shewanella putrefaciens*, along with widely distributed bacterial strains like *Staphylococcus*, were detected at various salinity levels (Table 3), including highly alkaline Lake Salda (Fig. 6b). The abundances of cultivable viable bacteria were significantly higher on the MPs surfaces than in the water samples ($P < 0.05$) (Fig. 7).

Discussion

In the 11 lakes with salinity levels ranging from 0.25 to 192.69‰, the presence of MPs exhibited variations independent of salinity. The total concentrations of the different types of MPs in the lakes (ranging from 0.25 to 1.75 items/l) were relatively low compared to the results from urban areas worldwide (Malla-Pradhan et al., 2022; Mercy et al., 2023; Yin et al., 2019). For instance, registered MPs in Lake Phewa in Nepal ranged from 0.8 to 8 items/l (Malla-Pradhan et al., 2022) and in Lake Luruaco, Colombia, from 0 to 3.83 items/l (Rojas-Luna et al., 2023). However, the number of MPs items in our sampled lakes was higher

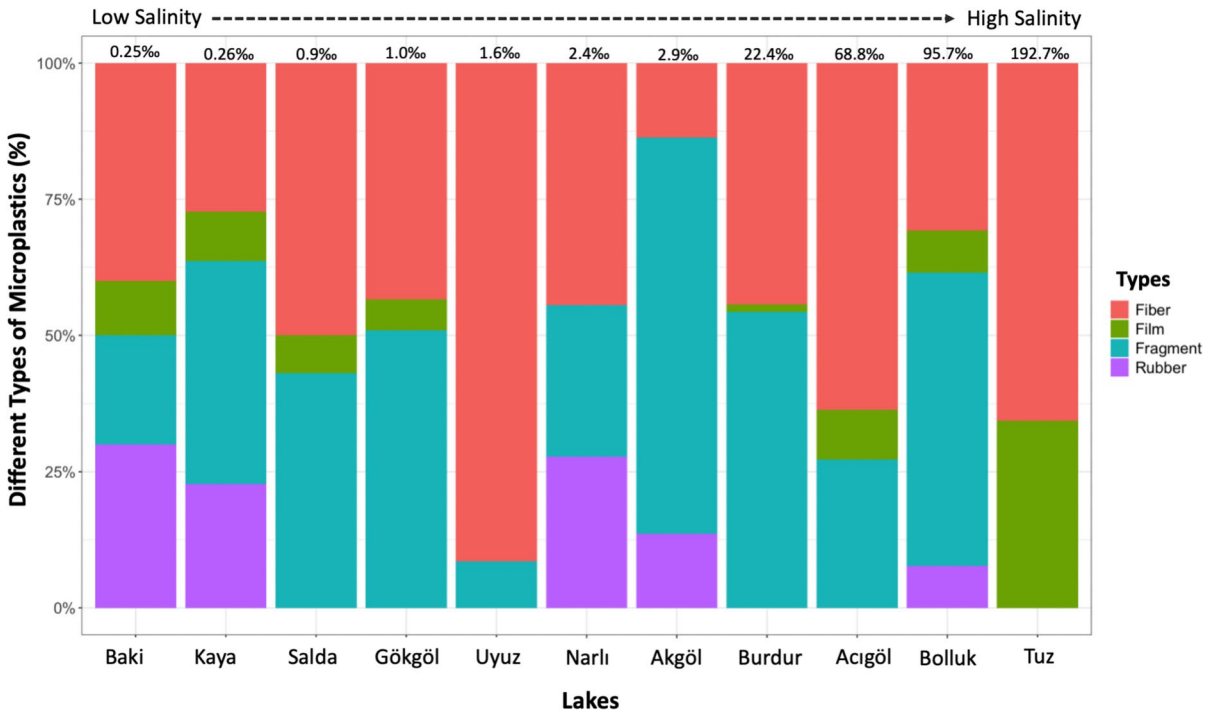


Fig. 3 Relative abundance of different types of MPs in inland lakes along a salinity gradient

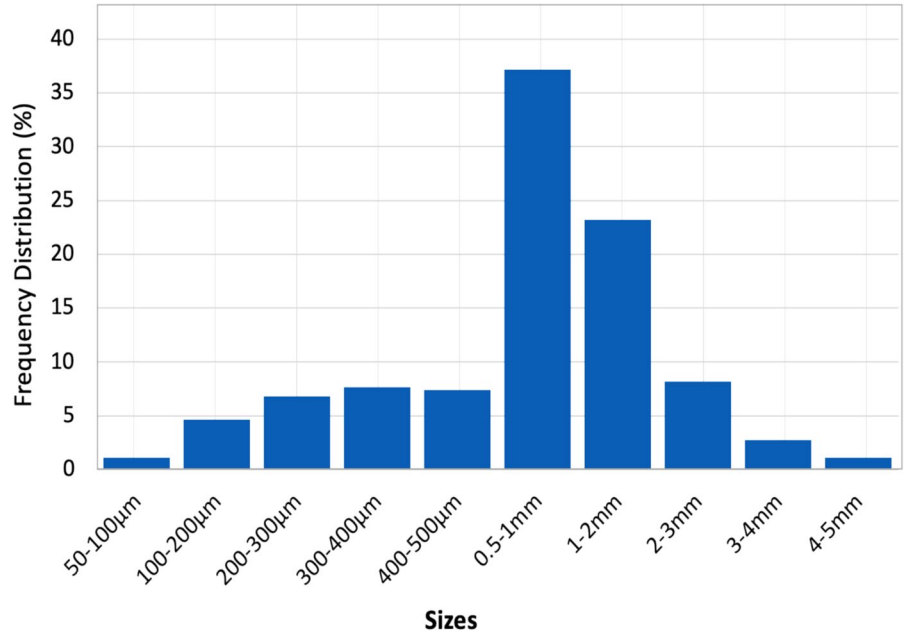


Fig. 4 Size distribution of microplastics

than those recorded in some other studies. In Lake Simcoe in Ontario, Canada, for example, MP abundance was in the range 0.4–1.3 items/m^3 (Felismino et al., 2021). Additionally, Lake Chiusi, Italy, demonstrated concentrations of 2.68 to 3.36 items/m^3, and Lake Bolsena, Italy, held 0.82 to 4.42 items/m^3

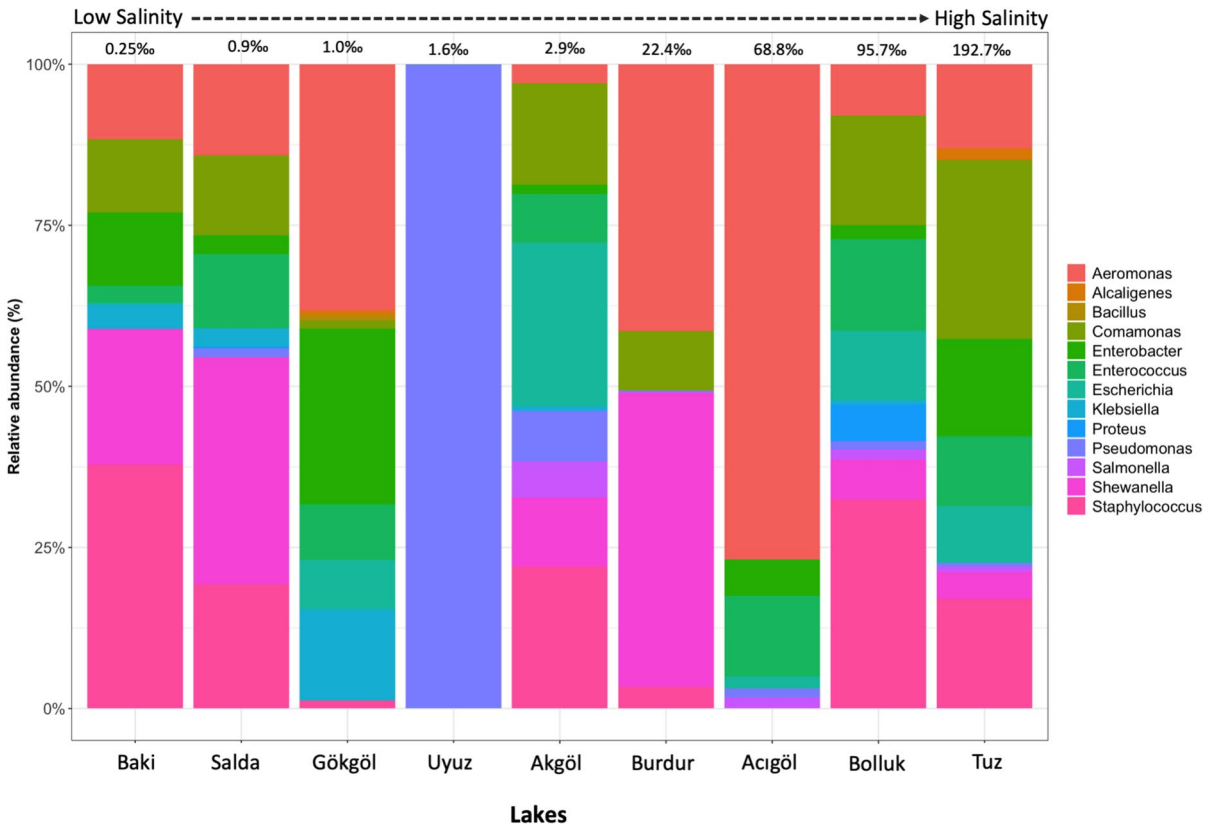


Fig. 5 Relative abundance of bacterial genera in MP biofilms sampled in inland lakes along a salinity gradient

(Fischer et al., 2016). Comparatively, Lake Kallavesi in Finland had concentrations ranging from 0.037 to 0.66 items/m^3 (Uurasjärvi et al., 2020). It is noteworthy that the lakes with the highest MPs concentrations in our study, Lake Burdur (1.75 items/l), Lake Salda (1.45 items/l), and Lake Tuz (0.8 items/l) (Table 2), are prominent tourist destinations located in close proximity to industrial activities (Demir, 2014; Kapan, 2016; Keskin, 2017; Malygina et al. 2021; Temurçin & Tozkoparan 2021). The association between elevated MPs levels and tourism, as well as industrial influence, underscores the potential impact of human activities on plastics pollution. Similarly, in lakes Gökgöl (1.33 items/l), Akgöl (1.10 items/l), and Uyuz (0.88 items/l), where substantial MPs concentrations were detected, the disposal of single-use plastics by visitors and from agricultural and industrial runoff in the region has been argued to be relevant contributors of plastics pollution (Arslan, 2019; Eken et al., 2006; Özdemir & Bahadır, 2009). Furthermore, at two popular tourist destinations, Lake Narlı and Lake Kaya, MP concentrations were found to be relatively high, with 0.45 and 0.55 items/l, respectively. These high abundances of MPs can be associated to recreational activities such as swimming with clothes, amateur fishing, and road usage around the lakes (Acar & Altaş, 2017; Doğanay & Zaman, 2001; Kaya et al., 2015). This further emphasizes the role of recreational and local practices in MPs contamination. In contrast, lakes Baki, Acıgöl, and Bolluk exhibited lower microplastic concentrations (0.25, 0.28, and 0.33 items/l, respectively), likely reflecting that the lakes are located in less populated areas with minimal industrial and agricultural activity (Bağdatlı & Nazari, 2022; Güçlü & Küçük, 2012). The presence of fibers and fragments in relatively high concentrations reveals formation of secondary MPs from the fragmentation of larger plastics as in other aquatic environments (Alirezazadeh et al., 2023; Cox et al., 2021; Kaliszewicz et al., 2020; Nava et al., 2023; Negrete Velasco et al., 2020; Xia et al., 2020).

Table 3 Bacterial strains found in the study lakes

	Bacterial Strains	Baki		Kaya		Salda		Gökgöl		Uyuz		Narlı		Akgöl		Burdur		Acıgol		Bolluk		Tuz	
		W	MP	W	MP	W	MP	W	MP	W	MP	W	MP	W	MP	W	MP	W	MP	W	MP	W	MP
Proteobacteria	*Aeromonas hydrophila/punctata*	*	*	*			*		*	*				*	*	*	*	*	*	*	*	*	*
	Alcaligenes spp.																						*
	Comamonas aquatica	*	*	*		*	*		*	*	*	*		*	*	*	*	*		*	*	*	*
	Enterobacter aerogenes		*	*			*		*	*				*	*	*		*	*		*	*	*
	Escherichia coli	*	*	*					*		*			*	*						*	*	
	Klebsiella sp.		*	*			*		*					*	*			*			*	*	*
	Proteus spp.						*									*		*			*		
	Pseudomonas spp.	*									*												
	Salinivibrio proteolyticus															*							
	Salmonellathypi/thypimirium		*				*			*					*		*		*		*	*	
	Shewanella putrefaciens	*	*	*		*	*			*	*	*		*	*	*	*	*		*	*	*	*
	Vibrio spp.															*							
Firmicutes	*Bacillus flexus*													*									
	Bacillus marisflavi								*							*							
	Bacillus spp.								*														
	Enterococcus faecalis	*	*	*			*		*	*	*			*	*	*		*	*		*	*	*
	Staphylococcus warneri										*												
	Staphylococcus aureus	*	*				*		*	*	*			*	*	*	*	*		*	*	*	*

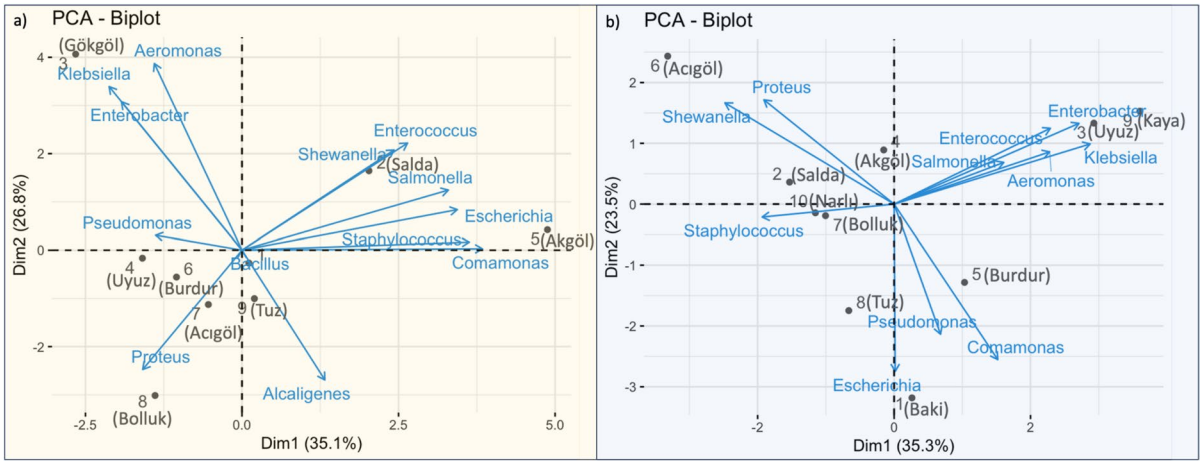


Fig. 6 Principal component analysis (PCA) based on grouping of lakes as **a** MPs-attached bacteria and **b** bacteria in lake

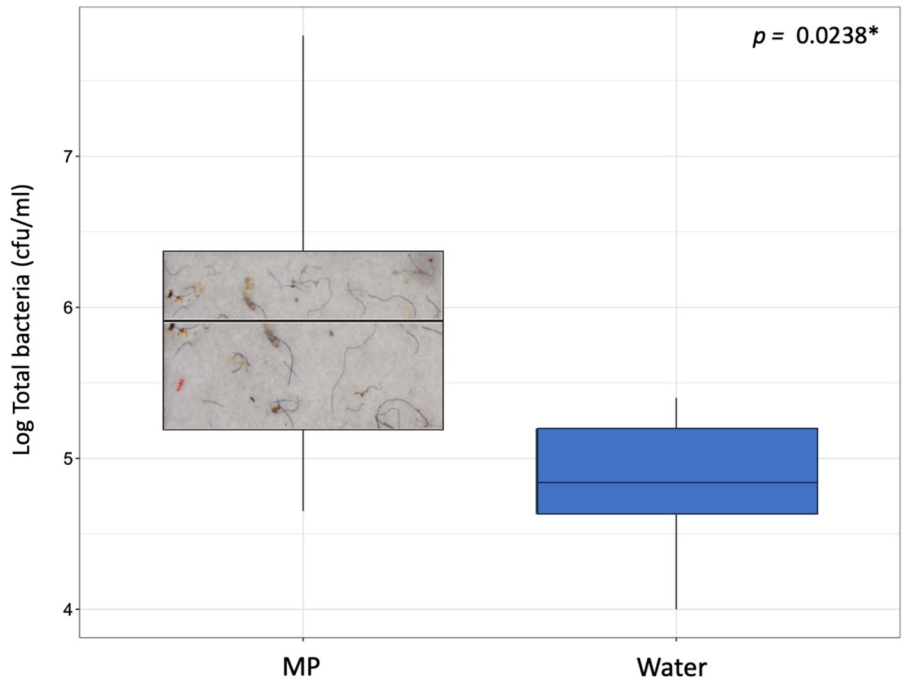


Fig. 7 Boxplot comparing total bacterial abundance between MPs-attached bacteria and bacteria in lakes

The prevalence of identified colors in the current study (black 52%, blue 22%, and transparent 13%) aligns with the findings of previous studies (Wang et al., 2018; Yin et al., 2019). The higher concentration of blue-colored microplastics in aquatic environments might be attributed to faster photoaging of bluish microplastics compared to red or yellow plastics, resulting from short-wavelength light absorption with higher energy (Zhao et al., 2022). The resemblance of transparent, white, and blue-colored MPs with the primary food source of pelagic fish such as plankton raises concern about the potential of intentional ingestion by fish (Boerger et al., 2010; Nadal et al., 2016).

In aquatic ecosystems, bacteria typically exist in a planktonic state, while MPs serve as a substratum for bacterial attachment, fostering their growth and enabling the development of complex microbial

communities (Caruso, 2015; Fabra et al., 2021; Ridall et al., 2023). Nevertheless, variations can also arise due to environmental factors (Oberbeckmann et al., 2014) such as salinity. However, we did not find a significative correlation of MPs concentrations in response to the explored salinity gradient.

We observed similar counts of bacterial strains (CFU/ml) in the surrounding water and the MPs surfaces (Table 3). However, we noted differences in the presence of viable cultivable species in saline lake water compared to those on the MPs. Our research shows discernible changes in cultivable viable bacterial species within the biofilm on MPs surfaces along the salinity gradient (Fig. 5). This highlights the dynamic interplay between MPs and bacterial communities across different aquatic environments, suggesting a complex relationship influenced by salinity. For instance, in very high-saline Lake Tuz, the plastisphere bacteria community was dominated by *Klebsiella* sp. (Fig. 5), which is a potential pathogen for nosocomial infections and frequently found in soil, surface water, sewage, and industrial effluent (Podschun & Ullmann, 1998). *Klebsiella* sp. can degrade and utilize polyethylene as a carbon source through the enzymes that it produces (Awasthi et al., 2017). The detection of *Alcaligenes* sp. in the lake with the highest salinity, Lake Tuz (Fig. 6), demonstrates the adaptability of these organisms to a high-saline environment. Despite the considerable distance from the sea to the lake, the finding of viable bacterial strains such as *Vibrio* and *Shewanella* in lakes Burdur and Salda on MP surfaces raises concern about their origin and adaptation mechanisms. These strains are commonly associated with marine ecosystems or high-salinity environments (Dillon et al., 2009; Swan et al., 2010; Hawley et al., 2014). *Vibrio* has been studied regarding their versatile adaptability to a wide range of salinity and temperature conditions (Ramalingam & Ramarani, 2006; Alam et al., 2009). Furthermore, the occurrence of the halotolerant *Proteus spp*. in highly saline Lake Bolluk emphasizes the significance of salinity gradients in shaping bacterial community composition and diversity. Our findings demonstrate the complexity of microbial dynamics in lacustrine environments and accentuate the need for further investigation into the factors driving bacterial distribution and adaptation in inland waters. Additionally, their presence serves as an indicator of fecal contamination (Drzewiecka, 2016). *S. aureus,* characterized as a halotolerant eubacterium (Vijaranakul et al., 1995), was, however, detected not only in Lake Tuz but also in freshwater Lake Baki. *S. aureus,* which exhibits adaptational mechanisms to tolerate varying salinity levels and displays antimicrobial resistance, poses a potential threat to public health (Olson et al., 2002; Perveen et al., 2023) through human utilization of these waterbodies. In addition to *S. aureus*, the widely distributed pathogenic bacteria *Aeromonas hydrophyla*, *Comamonas aquatica*, and *Enterobacter aerogenes* occurred on the surfaces of the MPs at all salinities (Table 3). Biofilm on the surface of MPs has mostly been investigated in marine and freshwater (Harrison et al., 2014; Oberbeckmann et al., 2014, 2016; Hoellein et al., 2014; McCormick et al., 2014; Amaral-Zettler et al., 2015; Lagarde et al., 2016; Oberbeckmann & Labrenz, 2020), while little is known about biofilm in saline inland lakes.

Due to their small size, buoyancy, and attractive colors, microplastics pose a risk of ingestion by various aquatic organisms. Gobies, a family of bony fish, have been observed to consume MPs directly due to their visual similarity to prey (Liu et al., 2020). Birds such as auks, petrels, gulls, ducks, and geese may mistakenly ingest microplastics, potentially impacting their health (Robuck et al., 2022). It is widely acknowledged that primarily shape, size, and taste determine the ingestion by aquatic organisms of microplastics (Aguirre-Mertinez et al., 2023; Yin et al., 2024). For instance, the absence of MPs in small-sized zooplankton such as rotifers highlights a size-selective uptake phenomenon (Başaran Kankılıç et al., 2023). Filter-feeding birds like flamingos could also accidentally consume MPs as they resemble their primary food source, plankton (Alfonso et al. 2020). The dominant sizes of MPs in our study, <2 mm, concur with previously reported sizes (Fischer et al., 2016; Wang et al., 2018; Kaliszewicz et al., 2020). The presence of MPs as a transport vector for pathogens (Agathokleous et al., 2021) amplifies their potential to inflict harm on aquatic ecosystems (Thacharodi et al., 2024). Increased heterotrophic activity due to the development of microplastics-associated biofilms has been shown to alter the carbon cycling in aquatic ecosystems (Arias-Andres et al., 2018). Additionally, the toxic additives in plastics can negatively affect bacterial growth (Sheridan et al., 2022). As vectors for microorganisms, MPs may also

facilitate potential pathogenicity and antibiotic resistance (Di Pippo et al., 2022), thereby posing an environmental health risk. In addition, the role of MPs in aquatic ecosystems as a new substrate for bacterial growth impacts the metabolism as plastic leaching can provide energy for bacterial growth (Romera-Castillo et al., 2018). Although our study does not directly address the impact of MP-associated denitrifying bacteria, the presence of denitrifying species like *Pseudomonas* and *Alcaligenes* in the sampled lakes may significantly contribute to global warming (He et al., 2024). Consequently, lakes facing multiple stressors, including climate change, salinization, and eutrophication, are now also confronted with the emerging challenge of MPs. Considering that biofilm morphology is influenced by environmental factors such as water organic content, salinity, and dissolved oxygen concentration (Nguyen et al., 2022), no location worldwide is immune to the impact of plastics pollution (Nava et al., 2023). In addition, socioeconomical activities play a critical role in the reduction of microplastics, making site-specific mitigation measures essential (Tavşanoğlu et al., 2024).

Conclusion

Our findings suggest that MPs play a key role in the development of bacterial communities with potential drastic environmental and health effects.

Furthermore, it indicates the potential role of the development and dispersal of bacteria with a significantly different composition compared to those in lake water. Moreover, the presence of pathogenic bacteria was ubiquitous in MPs along the salinity gradient. This emphasizes the critical role that MPs might represent in driving the dispersion and drastic compositional changes of benthic bacteria, including pathogens, in inland waters. It further suggests that this contrasting composition and abundance of bacteria in buoyant and colorful particles of MPs increase the bioavailability and potential consumption at higher trophic levels, thus augmenting the risk of environmental and health effects of not only microplastics, but also pathogenic bacteria.

Although our study has limitations, regarding, for instance, surface properties and the polymer composition of MPs, the results demonstrate the presence of organisms with high adaptability to saline environments associated with MPs. Additionally, the occurrence of microorganisms, especially pathogenic bacteria, on the surface of MPs in the investigated lakes indicates that MPs may act as a substrate for such bacteria, creating an ecological niche that may potentially compromise ecosystem environmental health. Studies utilizing high-throughput sequencing to assess the composition and diversity of bacteria on MPs should be conducted to better understand the role of the plastisphere in inland water bodies affected by MPs contamination. Further research in diverse environments is imperative to enhance our understanding of the sources and impacts of MPs.

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Author contributions Kerem Gökdağ was involved in investigation, formal analysis, writing—original draft. Tülay Pekmez helped in formal analysis. Gülçin Akca contributed to formal analysis, methodology, writing—review and editing. Mustafa Korkmaz took charge of investigation. Juan Pablo Pacheco helped in investigation, writing—review and editing. Gökben Başaran Kankılıç contributed to formal analysis, writing—review and editing. Cemreay Dede was involved in investigation. Korhan Özkan, Meryem Beklioğlu, and Zuhal Akyürek helped in writing—review and editing. Erik Jeppesen contributed to funding acquisition, writing—review and editing. Ülkü Nihan Tavşanoğlu took charge of supervision, conceptualization, methodology, writing—review and editing. All authors read and accepted the final manuscript.

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Data availability Data can be obtained upon request from the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval Not applicable.

Consent for participate Not applicable.

Consent for publication Not applicable.

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