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Occurrence of polycyclic aromatic hydrocarbons, microplastics and biofilms in Alqueva surface water at touristic spots

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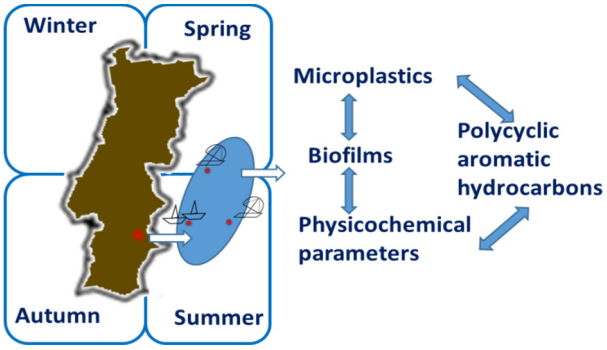
HIGHLIGHTS

- First study on microplastics (MPs) in Portuguese inland freshwaters (Alqueva).
- MPs are more often colonized by polymicrobial biofilms (phytoplankton, bacteria and fungi) than natural materials.
- Listed as priority pollutants by USEPA, polycyclic aromatic hidrocarbons (PAHs), were detected in surface water samples.
- Potential use of bioremediation mediated by biofilm communities.

GRAPHICAL ABSTRACT



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ABSTRACT

Freshwater pollution is a huge concern. A study aiming to evaluate physico-chemical characteristics, microbiota, occurrence of two groups of persistent environmental pollutants with similar chemical properties (polycyclic aromatic hydrocarbons- PAHs and microplastics - MPs) in Alqueva's surface water was performed during 2021. Water samples were collected at three spots related to touristic activities (two beaches and one marina) during the Winter, Spring,

Abbreviations: Ace, acenaphthene; Acy, acenaphthylene; Ant, anthracene; ATR, Attenuated total reflection; BaP, benzo[*a*]pyrene; Bbf, benzo[*b*]fluoranthene; BghiP, benzo[*ghi*]perylene; BkF, benzo[*k*]fluoranthene; CFU, colony-forming units; Chr, chrysene; COVID-19, coronavirus disease 2019; DahA, dibenz[*a*,*h*]anthracene; DLLME, dispersive liquid–liquid microextraction; DO, Dissolved Oxygen; EU, European Union; Flt, fluoranthene; Flu, fluorene; FTIR, Fourier transform infrared; GC–MS, gas chromatography mass spectrometry; HDPE, High density polyethylene; HMW, high molecular weight; Ind, indeno[1,2,3-*cd*]pyrene; LDPE, Low density polyethylene; LMW, low molecular weight; MMW, medium molecular weight; MPs, microplastics; Nap, naphthalene; NP, nanoplastics; PA, Polyamide; PAHs, Polycyclic aromatic hidrocarbons; PBS, phosphate buffer saline; PCA, Plate Count Agar; PE, polyethylene; PET, polyethylene terephthalate; PFA, paraformaldehyde; Phe, phenanthrene; PP, polypropylene; PPE, personal protection equipment; PS, polystyrene; PVC, Polyvinyl chloride; Pyr, pyrene; TOC, Total organic carbon; USEPA, United States Environmental Protection Agency; WHO, World health organization.

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Polycyclic aromatic hydrocarbons
Biofilms

Summer and Autumn seasons. In addition, the presence of biofilms on plastic and natural materials (stone, wood/ vegetal materials) were assessed and compared.
Water quality based on physicochemical parameters was acceptable with a low eutrophication level. PAHs concentration levels were lower than the standard limits established for surface waters by international organizations. However, carcinogenic compounds were detected in two sampling locations, which can pose a problem for aquatic ecosystems. PAHs profiles showed significant differences when comparing the dry seasons with the rainy seasons, with a higher number of different compounds detected in Spring. Low molecular weigh compounds, usually associated with the atmospheric deposition and petroleum contamination, were more prevalent.
MPs were detected in all samples except one during the Winter season. The polymers detected were poly(methyl-2-methylpropenoate), polystyrene, polyethylene terephthalate, polyamide, polypropylene, styrene butadiene, polyvinyl chloride and low /high density polyethylene with the last being the most frequent. Biofilms were more often detected on plastics than on natural materials. In addition, biofilms detected on plastics were more complex with higher microbial diversity (*e.g.*, bacteria, fungi/yeast and phytoplancton organisms) and richer in extrapolymeric material. Based on morphological analysis a good agreement between microbiota and microorganism present in the biofilms was found. Among microbiota were identified microorganisms previously linked to plastic and PAHs detoxification suggesting the need for further studies to evaluate the viability of using biofilms as part of a green bioremediation strategy to mitigate water pollution.

1. Introduction

In Europe, industries engaged in plastic lifecycle from production to recycling represents a value-chain that employs over 1.5 million people (Plastics Europe, 2021). Plastics are part of our daily life due to an affordable price combined with superior chemical properties such as toughness, lightness and hydrophobicity that, unfortunately turns plastics so resistant to degradation (Rai et al., 2021). The mandatory use of personal protection equipment (PPE) during the ongoing coronavirus disease 2019 (COVID-19) pandemic increased the accumulation of plastic waste (Adyel, 2020) and MPs in water bodies (Aragaw et al., 2022). Only 21 % of plastic is recycled or incinerated, while the rest is weathered and broken down into smaller particles known as microplastics (MP, <5 mm) and nanoplastics (NP, <0.1 mm), affecting and being accumulated into organisms, soil and water (Yuan et al., 2020).

Most of the studies focus on MPs occurrence in marine waters. Marine and freshwaters are distinct in dimension, osmolarity and physical forces involved in mixing/transport that will influence the type and size of M/NPs. The distribution of MPs in Portuguese waters is poorly characterized with only four studies published, to the best of our knowledge, on this topic (Rodrigues et al., 2018; Rodrigues et al., 2019; Guilhermino et al., 2021; Sá et al., 2022). The study conducted in Antuã river showed that the water column, more than the riverbed, was severely affected by MPs contamination, being polyethylene (PE) and polypropylene (PP) the main sources (Rodrigues et al., 2018). The study performed in Lis river identified the most abundant polymers in water (PE, polystyrene (PS) and polyacrylate) and sediments (polyethylene terephthalate (PET) and polyacrylate) (Sá et al., 2022). The study in Douro estuary, highlighted the link between human activities and river contamination by MPs, as well as MPs impact on fish larva (Rodrigues et al., 2019); whereas the third study in Minho river focused on MPs distribution in different fish species (Guilhermino et al., 2021). The four studies were conducted on or include areas under tidal influence supporting the need for studying M/NPs occurrence in inland freshwater bodies. Freshwaters are of crucial importance since life in general and human beings in particular are highly dependent on it for drinking and food production. This fact shows that freshwater could function as a vehicle for MPs toxicity through all levels of the ecosystem (Eerkes-Medrano et al., 2015). Although it has been suggested that toxicity is driven not only by the plastic forming units (monomers) but also by adsorbed pollutants (such as PAHs, pesticides, etc.) and associated microorganisms, little is known about the underlying mechanisms (Carbery et al., 2018).

PAHs are ubiquitous environmental pollutants that can exist in >100 different combinations resulting from processes of incomplete combustion of organic materials. Due to their toxicity and adverse effects, 16 PAHs were designated as priority pollutants and regulated by USEPA. PAHs were also designated as priority hazardous substances by the European Commission, in the Directive on Environmental Quality Standards (Directive, 2008) being among the most widespread persistent organic pollutants in the water environment. They are of high environmental and human health concern, as they are toxic, susceptible to long-range atmospheric transport and able to bioaccumulate (Zhang et al., 2022). A major concern for human health, are the combined adverse effects, which are still largely unknown, related to their presence in environmental mixtures (Jarvis et al., 2014). PAHs adsorbed to MPs represent a potential major threat to many aquatic organisms. This is due not only to MPs ingestion, but also to leaching of contaminants (*e.g.* PAHs) and other incorporated additives. Once ingested by aquatic organisms the lipophilic contaminants can accumulate in fatty tissues, posing a long-term risk (Mansilha et al., 2013; Schrank et al., 2019). Several PAHs are known to be deleterious to aquatic species, such as pyrene (Pyr) that exhibits toxicity even at low levels of exposure, and benzo[*a*]pyrene (BaP) that is recognized as carcinogenic and an endocrine disruptor compound (Zhang et al., 2016). The mechanisms responsible for the effects of small MPs on phenotypes and the extent to which effects of MPs are modified by genetic and environmental factors are yet poorly understood.

On the other hand, if PAHs and biofilms persist in the same environment the toxicity outcome is unpredictable. Biofilms are complex communities of multispecies microorganisms formed virtually on any available surface/interface. In the last years, biofilm related infections emerged as a public health concern. Biofilms function as a bacterial virulence factor being associated to emergence of antibiotic resistant microorganisms able to cause life-threatening infections (Ramstedt et al., 2019). In the environmental context, biofilms might function as reservoirs of pathogenic microorganisms but a direct link to infection onset is not consensual (Kesy et al., 2019; Kaur et al., 2022). Biofilms could even be part of a solution for environmental concerns instead of a problem through bioremediation. Due to the high microbial biomass and ability to immobilize compounds, biofilms are especially suitable for the treatment of persistent pollutants. The enhanced gene transfer among biofilm microorganisms together with the reporting of bacterial genes responsible for PAHs and MPs degradation also facilitates bioremediation (Ping et al., 2014; Rajkumari et al., 2018).

Here we aim at characterizing surface water at three distinct locations at Alqueva linked to touristic activities in terms of physico-chemical properties, microbiota, PAHs and MPs. In addition, we investigated and compared plastic and natural materials colonization by microbial biofilms.

2. Materials and methods

2.1. Study site

The study was conducted at the biggest inland European artificial freshwater lake, Alqueva located in the southeast region of Portugal (Fig. 1). The dam that created this water reservoir with 83 km of length, an area of 250 km^2 and capacity for 4150 million m^3 (level 152 m) started operating

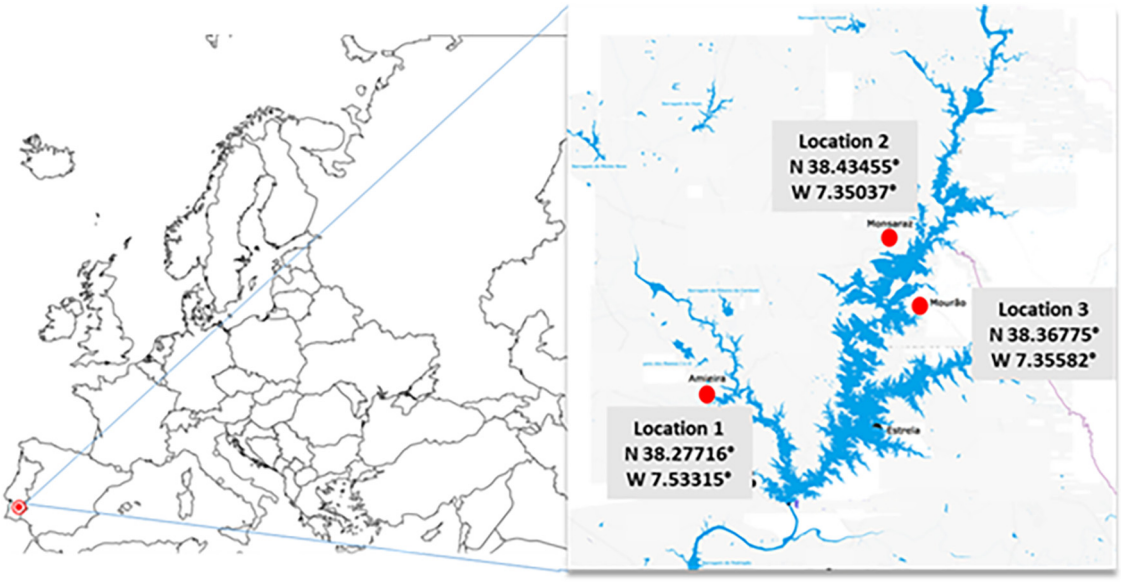

Fig. 1. Localization of Alqueva reservoir and sampling locations.

twenty years ago with the full capacity being reached for the first time in January 2010. Samples were taken at three spots related to ludic activities, one being a marina (N 38.27716° W 7.53315° - Location 1) and the other two fluvial beaches (N 38.43455° W 7.35037°- Location 2 and N 38.36775° W 7.35582°- Location 3) during 2021, once per season (Winter, Spring, Summer and Autumn). The levels of the dam during the Winter, Spring, Summer and Autumn sampling campaigns were of 150.31, 150.13, 148.96 and 148.27 m, respectively.

2.2. Sampling

Surface water samples were collected using adequate containers for PAHs, physical and chemical parameters determination, each season, over the course of one year (2021). Samples were transported in refrigerated containers to the laboratory and either analysed within 24 h or further processed and stored at 4 °C for posterior analysis according to ISO 5667-3 (ISO 5667-3, 2018).

For microbiologic analysis, samples were collected as previously described with slight modifications (Nascimento et al., 2016). Briefly, one liter of superficial water was collected at each location using a sterile glass bottle. Samples were transported in refrigerated containers protected from light and processed upon arrival to the laboratory. A similar procedure was adopted for microplastics sampling with the following differences 10 L of superficial water were collected in non-sterile bottles. Samples were transported and stored at 4 °C until further analysis.

For biofilm analysis, stone, wood or vegetal material and plastic particles up to 10 mm diameter (mesoplastics) were collected, flashed with water, transferred to glass vials containing 4 % paraformaldehyde (PFA) in 0.1 M phosphate buffer saline pH 7.4 (PBS), protected from light and transported in refrigerated containers to the laboratory. Samples were stored at 4 °C in 1 % PFA until further processing.

2.3. Physical and chemical parameters

Physical and aggregate properties of the samples were evaluated to determine water quality on all study sites. Temperature was measured on site using a probe (Traceable, Thermo Fisher, Waltham, MA, USA). pH (Mettler Toledo Seven Compact, Columbus, OH, USA) and conductivity (Crinson Conductimeter GLP31) were measured by electrometric methods; and turbidity by nephelometric method (HACH, CO, USA). For Total Dissolved Solids (TDS) quantification, 1000 mL of the sample were filtered using a glass fiber filter with 2.0 μm pore diameter (Whatman, Maidstone, UK), evaporated to dryness and its weight was monitored until a difference between two consecutive measurements was inferior to 0.5 mg.

Nutrient analysis was performed to access eutrophication and pollution levels. Nitrates (NO_3^-), ammonium (NH_4^+), nitrites (NO_2^-), silica (SiO_2) and phosphates (PO_4^{3-}) were determined by UV–visible spectrophotometry (HITACHI U-1100, Tokyo, Japan); Dissolved Oxygen (DO) was determined using the azide modification method according to official recommended methods(American Public Health Association et al., 2005).

Total Organic Carbon (TOC) was analysed by purging/persulphate digestion method, using Hach LCK385 cuvette test, according to manufacturer's instructions (HACH).

2.4. Polycyclic aromatic hydrocarbons (PAHs)

PAHs were analysed by a methodology based on dispersive liquid–liquid microextraction (DLLME) followed by gas chromatography mass spectrometry (GC–MS), in a Shimadzu GCMS-QP2010 gas chromatograph mass spectrometer equipped with an auto injector AOC5000 (Shimadzu Corporation, Kyoto, Japan), as previously described (Borges et al., 2018).

A PAH standard mix solution of 16 USEPA priority PAHs (naphthalene, Nap; acenaphthylene, Acy; acenaphthene, Ace; fluorene, Flu; phenanthrene, Phe; anthracene, Ant; fluoranthene, Flt; pyrene, Pyr; benz[*a*]anthracene, BaA; chrysene, Chr; benzo[*b*]fluoranthene, BbF; benzo[*k*]fluoranthene BkF; benzo[*a*]pyrene, BaP; dibenz[*a*,*h*]anthracene, DahA; benzo[*ghi*]perylene, BghiP; and indeno[1,2,3-*cd*]pyrene, Ind), each at 100 μg/L in dichloromethane, were purchased from Sigma – Aldrich (Steinheim, Germany). The surrogate standard was a mixture containing naphthalene –d8 (N-d8), acenaphthene-d10 (Ace-d10), phenanthrene-d10 (P-d10), chrysene-d12 (Ch-d12) and perylene-d12 (Per-d12), which was added to the samples before extraction and used as an internal standard. Stock solutions were used to prepare working standard solutions for calibration and spiking experiments.

Methanol, dichloromethane and acetonitrile were organic trace analysis grade Supra- Solv and were supplied by Merck (Darmstadt, Germany). Ultrapure water was highly purified by a Milli-Q gradient system (18.2 mW/cm) from Millipore (Milford, MA, USA).

2.5. Microbiology

Water samples were homogenized by inverting the recipient several times before filtration through membranes filters with 0.45 μm pore diameter (Merck-Millipore, Darmstadt, Germany) using a filtration slant

(Merck-Millipore). In all cases, 1 and 10 mL of the sample were filtrated. The membranes were then transferred either to non-selective or selective solid culture media and incubated at three different temperatures (30 °C, 37 °C and 44 °C) either for 24 h to 48 h (bacteria and yeast) or 5 days (fungi). Nonselective media was Plate Count Agar (PCA) and among selective media Sabouraud with cloranphenicol and MacConkey were used for yeast/ fungi and Gram-negative bacteria, respectively. Total culturable microorganisms were enumerated. Bacterial identification was performed using VITEK MS systems (bioMerieux). Briefly, a homogeneous microbial suspension was prepared from over-night cultures in 0.45 % sodium chloride solution adjusted to a turbidity of 0.5 McFarland (~1.5×10^8 colony-forming units (CFU)/mL). The microbial suspension further processed according to the manufacturer's instructions.

For yeast and fungi colonies features such as topography, colour, texture, diffusible pigments, hyphae were analysed. Microscopic mounts were assembled using tease mount or sellotape preparations and lactophenol cotton blue mount procedures. The identification was performed based on microscopic and macroscopic features of the colonies according to the literature (Samson et al., 2010).

2.6. Microplastics identification

In order to avoid contamination during sample processing cotton labcoats, gloves, glass and stainless-steel labware were used. In addition, samples were processed inside a flow chamber cabinet and a control filter was processed in parallel. Two liters of each sample were filtered through cellulose membranes filters with 0.45 μm pore size (Merck- Millipore), transferred to a glass Petri dish using stainstell forceps and allowed to dry at room temperature inside a desiccator. Filters were analysed under a Zeiss Axio Imager.A2m optical microscope (Carl Zeiss Microscopy, Oberkochen, Germany) using magnifications between 5× and 50× for the presence of plastic particles. These particles were transferred to uncoated calcium fluoride windows (ISP Optics, Latvia) and analysed by means of Fourier transform infrared (FTIR) microscopy with an iN10 and a Continuμm FTIR microscopes (both Thermo Scientific Nicolet, Waltham, MA USA). The iN10 microscope operates with an internal Globar source and was used in a single point ATR mode. The Continuμm FTIR, attached to the infrared beamline IRIS at the electron storage ring BESSY II of the Helmholtz Zentrum Berlin (Schade et al., 2002), was used in transmission mode to investigate particles with typical size of 10 μm, by use of infrared synchrotron radiation. The data were collected in the range of 650–4000 cm^{-1} using MCT detectors. The experimental spectra were compared to BRUKER 10.000+ IR-spectral database using OPUS 8.2 software (both Bruker Optik, Ettlingen, Germany) to identify the microparticles material.

2.7. Biofilms

For biofilm analysis three distinct materials (stone, wood/plant material and plastics) were prepared as previously described (José and Jordao, 2020). Briefly, fixative was removed, the sample was washed twice with PBS and post-fixed with 1 % osmium tetroxide for 1 h at room temperature in the dark. Samples were washed twice for 10 min with PBS and water before dehydration with 50 %, 70 %, 80 % and 95 % ethanol in water for 30 min and absolute ethanol twice for 30 min. The samples were allowed to dry at room temperature before being assembled on top of double-sided carbon tape (EMS), sputter coated with a 25 nm gold/palladium layer using a QISOT ES Sputter Coater (Quorum Technologies, Laughton, UK) and analysed under a JSM-7100F electron microscope (JEOL, Tokyo, Japan) or a Phenom ProX G6 (Thermo Scientific). The plastic polymer was identified as described in Section 2.6.

3. Results and discussion

Human presence and economic activities affect environmental pollution including water pollution. In this study, three sampling points located in an inland freshwater body related to ludic activities (direct or indirectly linked to tourism): a marina (Location 1) and two fluvial beaches (Locations 2 and 3) as shown in Fig. 1 were selected and monitored, once per season, during 2021.

3.1. Physico-chemical characteristics

3.1.1. General characterization of superficial water samples

The physical and chemical properties of water samples did not present remarkable differences throughout the study as shown in Table 1. The parameter registering the largest seasonal variations was temperature ranging from 14 °C (Winter) to 28 °C (Summer), as expected since surface water is more prone to be affected by environmental changes. pH followed the temperature variation with higher values in the Summer and lower in the Winter (7.8–9.0) (Serafim et al., 2006). For conductivity and TDS no correlation to seasons was established with values ranging between 320 μS/cm to 450 μS/cm and 200 mg/L to 320 mg/L, respectively.

The lowest DO concentration was registered during the Winter at 3.5 mg/L, which can be explained with the rise of water levels, as sediment, vegetation and other substances present on the shore can be dragged and mixed. The availability of organic matter can lead to microbial biodegradation decreasing DO concentrations (Kamp-Nielsen, 1974). This also can account for the higher concentration of silica since the main source on this type of reservoir is through sediment exchanges (Ning, 2010). The highest concentration of SiO_2 was observed at Location 1, during the Winter season.

Concentration of nitrogen compounds indicated nitrification was the main process in the nitrogen cycle, especially during the wet seasons, as nitrates (0.4 to 9.3 mg/L) and nitrites (<0.01 to 0.089 mg/L) were found to be present in a higher concentration than ammonium (<0.05 to 0.065 mg/L) (Hou et al., 2016). However, none of the above was found to surpass the maximum recommended values found on the Portuguese legislation for water quality (Decreto de Lei 236/98 de 1 de Agosto de 1998 do Ministério do Ambiente, 1998).

Phosphates concentrations remained low (mainly under 0.20 mg/L) at all locations throughout the study. This result together with the level of nitrogen compounds and the minimal fluctuations in TOC concentration, account for a low level of eutrophication (Wagner and Erickson, 2017). The fact that the studied spots are located in the south area of the reservoir and not in the close vicinity of intensive agriculture activity might had contributed to the observed result (Palma et al., 2010). Alqueva has been used to conduct several studies namely ecotoxicological studies *(*Palma et al., *2010;* Palma et al., 2014; Novais et al., 2019; S. Rodrigues et al., 2022). Although physicochemical parameters were determined in all studies, it is difficult to compare the obtained results due to differences in study designs (*e.g.,* different parameters, sampling locations, sampling seasons).

3.1.2. PAHs titration in surface water

Nine of the sixteen PAHs listed as priority pollutants by the USEPA were detected in the water samples: naphthalene (Nap), acenaphtylene (Acy), fluorine (Flu), phenanthrene (Phe), fluoranthene (Flt), pyrene (Pyr), benzo(*a*)anthracene (BaA), chrysene (Chr) and benzo(*k*)fluoranthene (BkF) (Fig. 2).

According to the ring number, PAHs can be classified into three classes: 2–3 rings, 4 rings, and 5–6 rings composition, which represent low (LMW), medium (MMW) and high (HMW) molecular weight hydrocarbons, respectively. The total concentration of PAHs was similar in the three sampling locations, with a mean value of 45.5 ± 8.5 ng/L, and the prevalence of Nap, Phe and Flt, which are LMW and MMW compounds, corresponding to 5.4 %, 50.2 % and 34.1 % of the total PAHs, respectively.

The presence of LMW PAHs in the water can be attributed to their high vapor pressure and water solubility, while the low concentration of HMW PAHs can be attributed to their lower water solubility and great tendency to adsorb onto solid phases. The high ratio of LMW PAHs compared to

Table 1
Physical and chemical properties of water samples.

	Location 1				Location 2				Location 3			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
T (°C)	15	18	23	22	14	19	25	23	17	20	28	24
pH	7.8	8.6	8.8	8.7	8.1	8.5	9.0	8.7	8.5	8.4	9.0	8.6
Turbidity (NTU)	5.5	2.1	1.7	2.4	2.2	0.88	2.7	4.3	3.4	3.1	3.1	2.6
Total dissolved solids (mg/L)	230	280	320	290	270	310	200	300	320	300	300	300
Conductivity[a] (μS/cm)	320	400	420	430	380	430	430	450	440	430	430	440
Total organic carbon (mg/L)	10	8.5	9.7	7.5	9.6	8.1	10	8.4	9.7	8.9	13	7.0
Dissolved oxygen (mg/L)	4.0	7.8	8.1	9.5	3.5	8.1	8.8	7.8	5.5	8.7	n.d[b]	8.4
Nitrites (mg/L)	0.050	0.058	0.014	< 0.01[c]	0.079	0.082	0.022	0.011	0.089	0.064	0.013	< 0.01[c]
Nitrates (mg/L)	3.1	1.5	1.0	1.0	5.6	0.4	0.8	9.3	3.6	3.4	0.8	0.7
Ammonium (mg/L)	<0.05[c]	0.065	<0.05[c]	<0.05[c]	<0.05[c]	<0.05[c]	<0.05[c]	<0.05[c]	<0.05[c]	<0.05[c]	<0.05[c]	<0.05[c]
Phosphates (mg/L)	0.27	< 0.20[c]	< 0.20[c]	< 0.20[c]	< 0.20[c]	< 0.20[c]	< 0.20[c]	< 0.20[c]	<0.20[c]	< 0.20[c]	< 0.20[c]	< 0.20[c]
Silica (mg/L)	4.5	1.2	< 0.43[c]	< 0.43**	3.2	0.97	< 0.43[c]	< 0.43[c]	1.3	0.64	< 0.43[c]	< 0.43[c]

[a] Determined at 20 °C.
[b] n.d. not determined.
[c] Quantification limit of the method.

HMW PAHs suggests that PAHs contamination in Alqueva may be of natural origin (petrogenic and biogenic) or a result of oil spillage from shipping, recreational and fishery boat activities (Santos et al., 2018; Stogiannidis and Laane, 2015).

PAHs analyses were performed in the four sampling campaigns, in Winter, Spring, Summer and Autumn. Regarding seasons variation, the highest concentrations were detected in Spring, in Locations 2 and 3, with 21.7 ng/L and 18 ng/L, values that are lower than the world health organization (WHO) limit of 50 ng/L for surface and coastal waters (Guidelines for Drinking-Water Quality,1998) and the standard limit of 100 ng/L for total PAHs of the new directive of European Union (EU) (Directive (EU), 2020).

The carcinogenic PAHs Chr and BkF were detected in Location 1, in Summer, and Chr was detected in Location 3, in spring, accounting for 9.0 % and 1.7 % of the total concentration of the 16-PAHs, respectively. The presence of carcinogenic compounds in water samples might generate a potential risk for the aquatic system, consequently affecting the food chain (Santos et al., 2018).

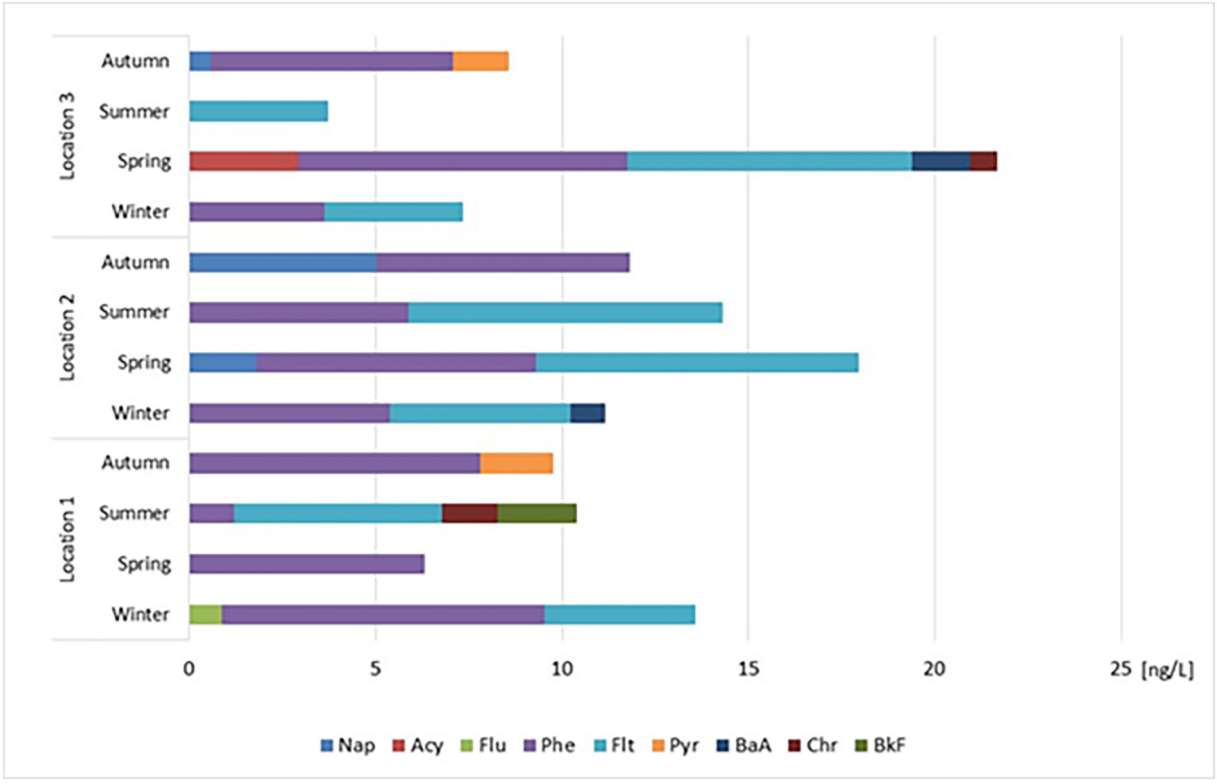

Fig. 2. Concentration of PAHs (mean values) in the studied samples.

3.2. Water microbiota

In order to characterize water microbiota total aerobic microorganisms were determined and identified (Table 2). Total aerobic microorganisms ranged between 91 CFU/mL (Location 1- Summer) and 359 CFU/mL (Location 3- Summer) being 204 CFU/mL the average value found during the study. The bacterial population predominates over the fungi/ yeast in good agreement with a previous study conducted by our group in freshwater lakes (Nascimento et al., 2016). Although it was not possible to identify a resident bacterial population in any location *Aeromonas sp.* among Gram negative bacteria and *Bacillus sp. among* Gram positive bacteria were the most often identified. Since our main goal was to characterize microorganisms' population and not evaluate water microbiological quality, we did not quantify coliforms and fecal enterococci. Nevertheless, *E. coli* was found in samples collected during the Winter and Spring seasons at location 3 that could be related to the presence of bovine cattle in pastures nearby.

Fungi and yeast were present in lower numbers being even absent at Location 3 and 2 during Winter and Spring, respectively. *Penicillium* and *Aspergillus* species were the most often identified probably due to their documented higher adaptability to aquatic environment that includes both natural and treated waters (Arroyo et al., 2019).

3.3. Microplastics occurrence

The MPs found in the samples were from a variety of polymers (Table 3) as reported in other studies (Laju et al., 2022). Plastic polymers used for food packing (PP, HDPE, PS), reusable bags and agriculture (LDPE), pipes (PVC), water and soft drinks bottles (PET) were among the most often identified in the analysed samples. This is an expected result since these polymers are known to be simultaneously among the polymers with higher demand in Europe (Plastics Europe, 2022) and major contributors for plastic waste (Miranda et al., 2020). The detection of acrylic could be explained by the current use of acrylic dyes and windows on boats. Polyamide (PA) is commonly used in clothes industry being its fibers a secondary plastic often found in freshwater (Eerkes-Medrano et al., 2015). The particles found were mainly fibers and for this reason, we did not categorize them. A typical polymer

Table 2
Characterization of water microbiota.

		Location 1	Location 2	Location 3
Winter	Bacteria	*Aeromonas salmonicida/bestiarum* *Aeromonas veronii* *Bacillus cereus* *Bacillus licheniformis* *Pantoea agglomerans* *Shewanella putrefaciens* *Serratia plymuthica*	*Aeromonas media* *A. salmonicida/ bestiarum* *A. veronii* *Bacillus cereus* *Erwinia rhapontici* *Pseudomonas cuarocienegasensis*	*A. media* *A. salmonicida/bestiarum* *Escherichia coli* *Lelliotia amnigena* Raoutella ornithinolytica
	Fungi and yeast	*Aspergillus fumigatus* *Aspergillus niger* *Aureobasidium pullulans* *Penicillim lusitanum* *Phoma sp*	*Chaetomium globosum* *Penicillium sp*	ND
	Total[a] (CFU/mL)	158	148	254
Spring	Bacteria	*Serratia plymuthica* *Lelliota amnigena* *Aeromonas sóbria* *Bacillus altidudinis/ pumillus* *Bacillus megaterium* *Bacillus circulans*	*Bacillus megaterium*	*Shewanella putrefaciens* *Aeromonas sobria* *E. coli* *Bacillus megaterium* *Bacillus cereus* group
	Fungi and yeast	*Cladosporium sphaerosphermum*	ND	*Arthrinius arundis* *Aspergillus fumigatus* *Cladosporium sp* *Chrysosporium sp* *Penicillium aurantigriseum* *Penicillium sp*
	Total (CFU/mL)	269	156	203
Summer	Bacteria	*Citrobacter braaki* *Chryseobacterium gleum* *Enterobacter cloacae* *Lelliotia amnigena* *Pseudomonas oleovans*	*Bacillus megaterium* *Exiguobacterium acetylicum* *Lelliotia amnigena* *Pseudomonas putida*	*Brevibacillus thermoruber* *Enterobacter cloacae* *Paenibacillus cineris* *Rahnella aquatilis* *Shingomonas paucimobilis*
	Fungi and yeast	*Aspergillus terreus* *Engyodontium sp* *Penicillium sp*	*Acremonium sp* *Aspergillus terreus* *Rhodotorula sp*	*Acremonium sp* *Aspergillus terreus* *Penicillium sp*
	Total (CFU/mL)	91	270	359
Autumn	Bacteria	*Aeromonas sobria* *Enterobacter cloacae* *Enterobacter kobei* *Pseudomonas mendocina* *Pseudomonas oleovans*	*Acinetobacter junii* *Aeromonas sobria* *Enterobacter cloacae* *Bacillus cereus* group	*Aeromonas sóbria* *Bacillus cereus group* *Bacillus clausii* *Bacillus megaterium* *Enterobacter hormaechei* *Exiguobacterium aurantiacum* *Klebsiella pneumoniae* *Pseudomonas oleovorans*
	Fungi and yeast	*Penicillium sp.*	*Aspergillus sp.* *Aspergillus terreus*	*Aspergillus terreus* *Aspergillus sp* *Fusarium sp.* *Penicillium sp* *Sarocladium sp.*
	Total (CFU/mL)	114	207	222

[a] Total aerobic microorganisms.

Table 3
Microplastics in water samples.

	Location 1	Location 2	Location 3
Winter	–	Acrylic[a] Polystyrene (PS) Low density polyethylene (LDPE)	Polyethylene terephthalate (PET)
Spring	Acrylic	Acrylic PS LDPE	Polyethylene (PE)
Summer	PS EUPRENE[b]	PS PE	PE
Autumn	Polyamide (PA) Polypropylene (PP)	LDPE PP PET	PA Polyvinyl chloride (PVC) LD/HDPE

[a] Poly(methyl- 2- methylpropenoate).
[b] Styrene butadiene.

particle found in water, the corresponding ATR-FTIR experimental and reference spectra are shown in Fig. 3. MPs were found in all sampling campaigns/locations with one exception: Location 1 during the Winter campaign. Although not being a quantitative study we could hypothesise that the detection of a lower number of polymers at Location 1 could, at least partially, be related to the fact that it is a restricted access area whereas the other two locations are freely accessed. Land-based sources account for 80 % of MPs found in the environment (Miranda et al., 2020). Among the sources that could contribute to the observed results are waterside leisure activities, recreational boating, agriculture practices, discharges from storm water drains, release of plastics from the soil among others (Miranda et al., 2020; Tanentzap et al., 2021). Once in the freshwater plastics distribution will be determined by polymer properties (*e.g.*, density, weight), exposure to weathering process (*e.g.*, UV, temperature), water flow and territory topography that could favour polutants concentration and persistence in a specific area. As could be observed in Fig. 1, the three sampling locations are located at distinct areas of lake experiencing differences in flow and topography that will influence polymer occurrence. On the other side, plastic degradation upon exposure to solar UV radiation is dose-dependent being exacerbated by higher ambient temperatures, higher humidity levels, and atmospheric pollutants (Andrady et al., 2019). In a region with a semi-arid Mediterranean climate classified as highly vulnerable to climate change and desertification due to, among other factors, reduced precipitation and increasing temperatures (Ferreira and Panagopoulos, 2014), plastic breakdown into micro and nanoplastics is expected to be enhanced.

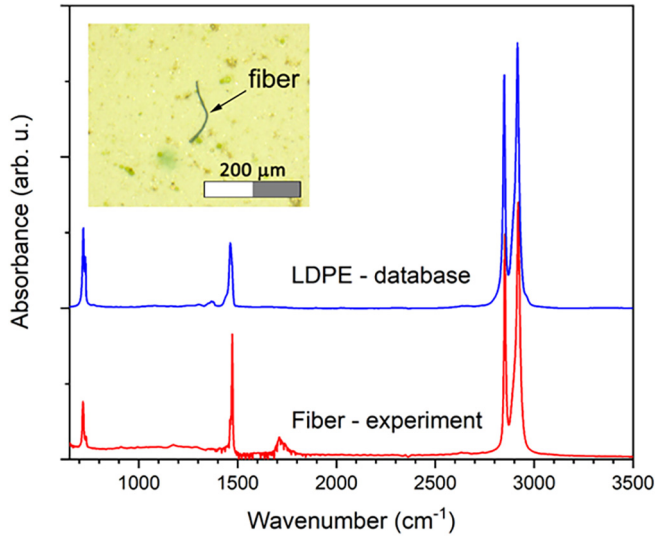

Fig. 3. ATR-FTIR spectra of the PE-fiber found at location 2 (Spring) and the LDPE reference (Entry No 71, Bruker Optics ATR-Polymer Library). The optical image of the investigated fiber on the surface of the microfilter is shown in the inset.

3.4. Biofilms

Finally, we analysed plastic particles (mesoplastics) that could originate microplastics by weathering and natural materials (stone, wood or vegetal materials) for the presence of biofilms. As could be observed in Table 4, it was possible to collect the three types of materials at all locations at all times with the exception of location 1. This result is in good agreement with the results of MPs occurrence previously presented (Table 3). In addition, the polymer of the different mesoplastics was always found during the same sampling campaign among the MPs polymers. Plastic polymers were more prone to biofilm colonization than natural materials (Table 4 and Fig. 4). Representative micrographs of biofilms assembled on LDPE, PS and PP are shown in Fig. 4A, B and C, respectively. The observed biofilms were formed not only by bacteria but also by fungi and phytoplankton organisms being clearly polymicrobial biofilms. The presence of high amounts of extracellular polymeric material is also evident as highlighted in the Fig. 4 C inlet. Stone samples, with the exception of one, were negative for biofilms (Fig. 4 D) whereas among wood/vegetal samples both positive (Fig. 4 E) and negative samples (Fig. 4 F) were found although the latest were more frequent. In addition, the biofilms found on wood/ vegetal samples exhibited a different phenotype being poorer in extracellular polymeric matrix content and microbial diversity as shown in Fig. 4 E inlet than those found on plastic polymers. Being a surface phenomenon biofilms are largely influenced by material characteristics such as chemical composition and surface topography. This could explain the differences observed not only between natural materials and plastic polymers (Oberbeckmann et al., 2018) but also between different polymers (José and Jordao, 2020) and polymers with different ages (Erni-Cassola et al., 2020; Huang et al., 2022).

The interaction between microplastics and biofilms are far from being fully understood. Nevertheless, fast plastic colonization by biofilms is known to alter plastic properties such as buoyancy and hydrophobicity (Rai et al., 2021) .In addition, some microorganisms are able to degrade plastics suggesting an environmentally friendly strategy to boost natural bioremediation and impact the cleaning of natural ecosystems without causing adverse effects (Shah et al., 2008; Yuan et al., 2020). The identification of several species of *Bacillus*, *Klebsiella pneumoniae*, *E. coli* among other species of microorganisms related to plastic degradation in these water samples lead us to speculate that they could colonize the polymers and contribute to their fragmentation and degradation as recently described for PP (Rana et al., 2022).

4. Conclusions

The superficial water in the studied area showed a low eutrophication level as assessed by physico-chemical parameters.

This study has provided valuable information on PAHs distributions and seasonal variations in superficial water samples from Alqueva. PAHs total

Table 4
Biofilms assembled on different environmental samples.

		Location 1	Location 2	Location 3
Winter	Stone	Negative	Negative	Positive
	Vegetal material	Negative	Negative	Negative
	Plastic	–	PS (positive) LDPE (positive)	–
Spring	Stone	Positive	Negative	Negative
	Vegetal material	Negative	Positive	Negative
	Plastic	–	PS (positive) PE (negative)	PE (positive)
Summer	Stone	Negative	Negative	Negative
	Vegetal material	Positive	Negative	Positive
	Plastic	–	PE (positive)	PE (positive)
Autumn	Stone	Negative	Negative	Negative
	Vegetal material	Positive	Negative	Positive
	Plastic	PP (positive)	PP (positive) PET (negative)	PVC (positive) HDPE (positive) LDPE (positive)

— material not found at the sampling spot.

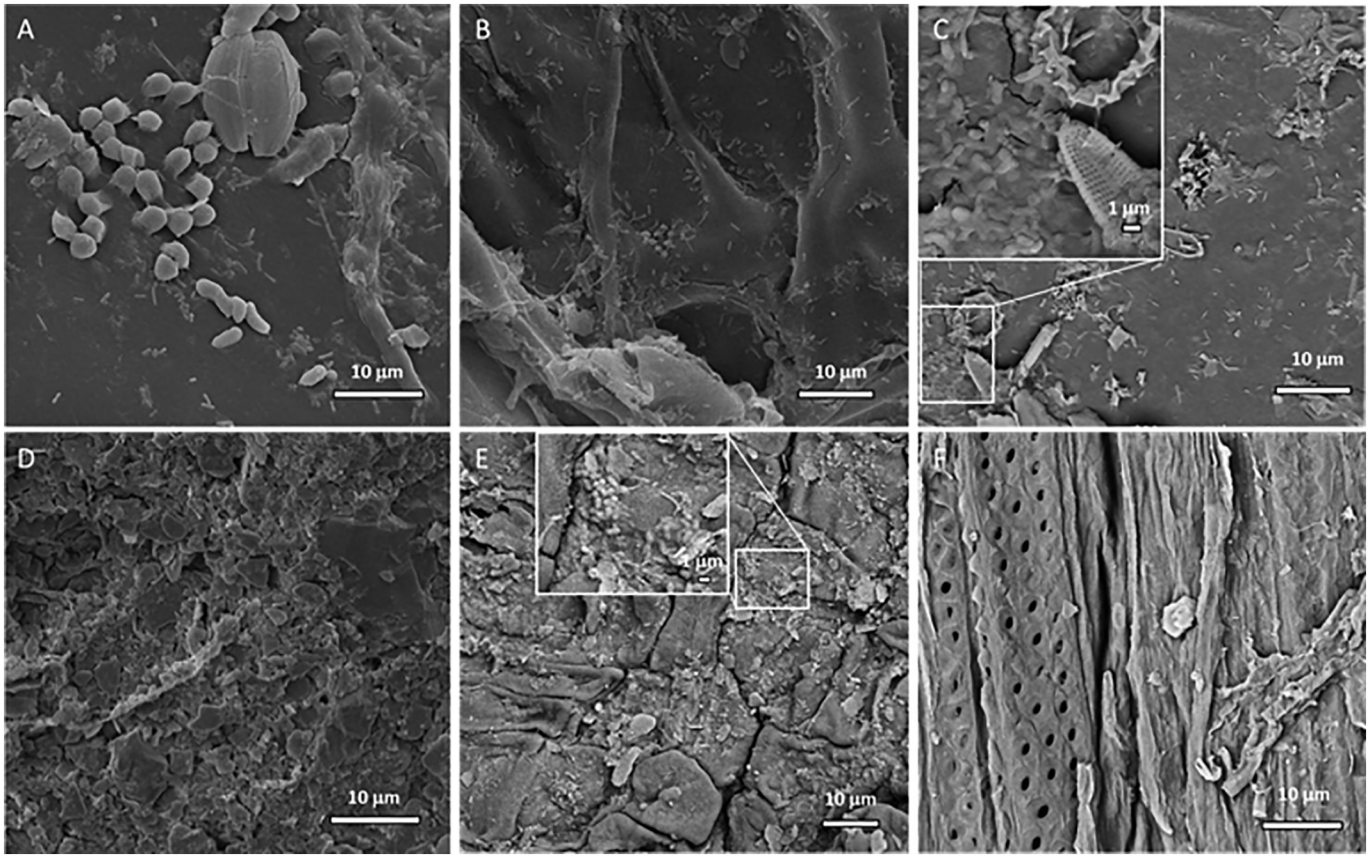

Fig. 4. Biofilms on environmental samples. Representative micrographs of biofilms assembled on different plastic polymers: LDPE (A), PS (B), PP (C). Natural materials such as stone (D) and vegetal samples (E, F) are also shown.

concentrations ranged from 3.7 to 21.7 ng/L, with a predominance of 2, 3 and 4-ring compounds, suggesting that the atmospheric deposition and petroleum contamination were the major input of PAHs in the sampling areas.

MPs were found at all locations during the study. The plastic polymers identified are used in different human activities in a daily basis what suggests that their presence in superficial water might be related to incorrect waste disposable. Plastic colonization by microbial biofilms was observed and should be studied to evaluate the viability of their use as a bioremediation strategy for environmental persistent organic pollutants.

CRediT authorship contribution statement

Conceptualization (LJ, CM), formal analysis (LJ, CM, AV), funding acquisition (LJ), investigation/ methodology (all authors), writing original draft (LJ, CM, AV, AR), review and editing (all authors).

Data availability

Data will be made available on request.

Declaration of competing interest

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

Maria Luisa Jordão on behalf of all authors.

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