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MICROPLASTICS IN A LOTIC FRESHWATER ENVIRONMENT: TYPOLOGY AND PROFILE OF OCCURRENCE ALONG JOUMINE STREAM, AFFLUENT OF THE ICHKEUL WETLAND (NORTHERN TUNISIA)

Rafika RAHMANI[1], Samir GHANNEM[1], Yasmina ZAHOUANI[2], Abdelhafidh KHAZRI[1], Sabri KANZARI[3], Essaid BILAL[4*] & Samir TOUAYLIA[1]

[1]Laboratory of environment biomonitoring, Life Sciences Department, Faculty of Sciences Bizerta, University of Carthage, Zarzouna, Tunisia.

[2]Laboratory of Integrated Physiology, Life Sciences Department, Faculty of Sciences Bizerta, University of Carthage, Zarzouna, Tunisia.

[3]National Institute of Research of Rural Engineering, Waters and Forests of Tunis, University of Carthage, Ariana, Tunisia

[4]Ecole Nationale Supérieure des Mines de Saint-Étienne, CNRS-UMR5600, Saint-Étienne, France, bilalessaid@gmail.com*

Abstract: The intensifying plastic production and poor management of its waste have led to a tremendous rise in the dumping into aquatic ecosystems, thus resulting in harmful effects on living organisms as well as biodiversity loss and wetland threat. The study aims to assess the availability of microplastics in a freshwater environment (the Joumine stream, Northern Tunisia) by determining their abundance, shape, type, color, and size in both water and sediment. Thirty samples were collected from five sites and analyzed for MPs using stereomicroscopy and FTIR spectroscopy. The average value of microplastics was found to be 8.87±3.94/L (in water) and 18.2±8.27/50g dry weight (in sediment). The most dominant form in all samples was fibers, while the color category varied according to the two considered matrices and sites. Microplastic particles in the samples ranged from 0.24 to 1.45 mm in length. Polypropylene and polyethylene were the identified types of polymers. Overall, the level of microplastic pollution along the Joumine stream was found to be relatively average compared to the global pollution level. It underlined the fact that MPs are widespread even in freshwater environments and provides a baseline for future surveys and management sustainable decisions.

Keywords: Freshwater environment; microplastics; FTIR spectroscopy; molysmology

1. INTRODUCTION

Plastic waste, particularly microplastics, is a growing environmental issue that concerns both scientists and the public (Koelmans et al., 2022). Microplastics are synthetic materials with high polymer content, solid particles less than 5 mm, insoluble in water, and non-degradable, making them easily introduced into and persistent in the environment (Sajjad et al., 2022). GESAMP (2019) has categorized microplastics into five groups, including fragments, foam, film, line, and pellets/granules. Microplastics are also categorized based on their origin as primary MPs from manufacturing (Browne, 2015) and secondary MPs resulting from degradation, which depend on factors such as UV exposure, temperature, polymer type, and additives (Bergmann et al., 2015). MPs are present in nearly all aquatic and terrestrial species that come into contact with them, regardless of their position in the food chain, due to their widespread presence, property, and large surface area (Xiang et al., 2022). MPs pose health risks and raise concerns about food safety and ecology (Lee & Fang, 2022). Multiple studies have documented the adverse effects of microplastics on organisms, which can result not only from the plastics themselves but also from the transfer of additives contained in plastic polymers (Chua et al., 2014; Wardrop et al., 2016) and the contaminants adsorbed to microplastics, such as metals, PAHs, PCBs, and organochlorine pesticides (Ashton et al., 2010). Freshwater systems, including sewage treatment plants, rivers, and isolated lakes, can serve as both sources and sinks for PMs. The amount of PMs in freshwater may vary significantly from that in seawater (Klein et al., 2018; Sulistiowati et al., 2023). Meijer et al., (2019) estimated that 80% of plastic emissions to the ocean come from 1000 rivers, with quantities ranging from 0.8 to 2.7 million tons per year. Despite its location along the Mediterranean Sea and limited water resources, Tunisia, suffering from a water resources shortage and endangered water potable safety, has few conducted studies on MPs, which is a significant data gap (Abidli et al., 2017). The investigation of MPs in both water and sediment is essential to understand their fate and potential impact on threatened aquatic ecosystems and biota (Ramos-Vázquez et al., 2024). Such lotic waterbody is complex and unstable which makes it hard to identify pollutant hotspots, fate, and target biota. However, consistent monitoring and implementation of mitigation strategies could lower the potential risks associated with these pollutants. This study aims to investigate the occurrence levels and typology of microplastics in water and sediment of the Joumine stream, among the main affluent of the Ichkeul wetland, with a focus on the longitudinal distribution profile from up to downstream. Such findings can be helpful tools in sustainability and strategic decisions targeting biodiversity, waterbodies, and Huma in preservation.

2. MATERIALS AND METHODS

2.1. Sampling and study area

The Joumine stream, the main tributary of the Ichkeul wetland, is located in northern Tunisia and forms an important natural and ecological continuum extending to the Bizerte lagoon through the narrow Tinja channel, with a wide connection to the Mediterranean Sea on the eastern side (Touaylia et al., 2016). This area experiences a sub-humid climate, with an average annual rainfall of approximately 600 mm (Dhib et al., 2021). The average temperature varies between 25°C in August and 8°C in January, and the region has an annual evapotranspiration potential of about 1600 mm. Since the construction of the Joumine Dam in 1984, the natural flow and functional integrity of the stream have been affected, especially during downstream flooding through the Mateur lowland towards the Ichkeul wetland, contributing to the lake’s salinization. Five representative sampling sites were chosen along the Joumine stream to capture the range of environmental conditions in the area, with three replicates collected per site during the spring, a season favorable for accessible and stable sampling conditions. The sampling sites are as follows:

- Sidi el Bechir (S1): 36°57′11.9"N, 9°27′41.95"E, rural area, 133 m elevation.
- Souidia (S2): 36°57′36.16"N, 9°31′48.94"E, rural area, 96 m elevation.
- El Arima (S3): 36°59′38.36"N, 9°36′52.03"E, rural area, 41 m elevation.
- Mateur (S4): 37°1′48.03"N, 9°39′47.62"E, rural area, 16 m elevation.
- Ichkeul (S5): 37°6′37.88"N, 9°41′58.82"E, wetland preserved area, 1 m elevation.

Sampling involved collecting 2L of surface water from stagnant areas at each site using glass bottles. Sediment samples were taken from the upper layer of the bottom sediment. GPS coordinates and site locations are provided in Figure 1.

2.2. Isolation of microplastics

For each water sample (n = 3), 1L was taken and filtered using a millipore vacuum pump onto 1.2 µm glass microfiber filters (Leslie et al., 2017). To ensure all potential microplastic particles were recovered, the water sample bottle was rinsed twice with ultrapure water and the rinse water was also filtered onto the same filter paper. Three quadrats (0.25 m × 0.25 m), with a distance of 1 m, were considered for sediment sampling, and natural debris (i.e. stone, wood) was removed. For each sampling site, the top layer of sediment (2–3 cm) was removed using a clean stainless-steel spatula and stored in closed glass containers for subsequent identification. The sediments were air-dried and 50 grams of dry sediment was mixed with high-density sodium chloride solution (140 g/L) in 1 L beakers and shaken vigorously for four days before the water was collected for filtration (Galgani et al., 2013). Before use, laboratory equipment utilized for sample preparation and extraction underwent two rinses with ultrapure water, while all liquids (including water and saline) underwent filtration with 1.2 µm pore size filters. To determine air contamination during laboratory work, Petri dishes with white paper were utilized. Control Petri dishes were positioned beside the other Petri dishes to be examined and analyzed for the presence of microplastics.

2.3. Microplastic observation and validation

The filters were scrutinized with care and the Petri dishes were constantly covered with aluminum foil to prevent contamination from airborne fibers while utilizing a stereo microscope with a calibrated and graduated (micrometric) eyepiece to sort and gauge the microscopic particles. Photos of the microplastics collected were captured using a 13 MP camera (Figure 2). A portion of the gathered PMs was manually extracted, specifically the largest particle,

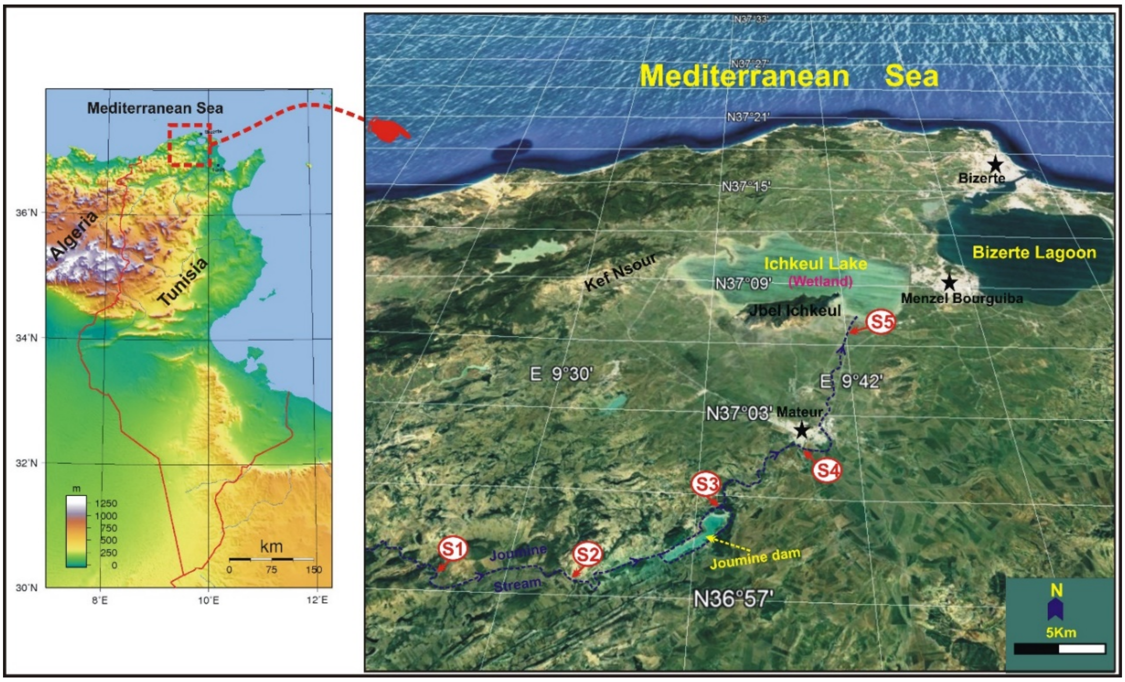

Figure 1. Map showing sampling sites with their GPS coordinates and altitudes. S1 (Sidi el Bechir): 36°57′11.9"N, 9°27′41.95"E, 133 m. S2 (Souidia): 36°57′36.16"N, 9°31′48.94"E, 96 m. S3 (El Arima): 36°59′38.36"N, 9°36′52.03"E, 41 m. S4 (Mateur): 37°1′48.03"N, 9°39′47.62"E, 16 m. S5 (Ichkeul): 37°6′37.88"N, 9°41′58.82"E, 1 m.

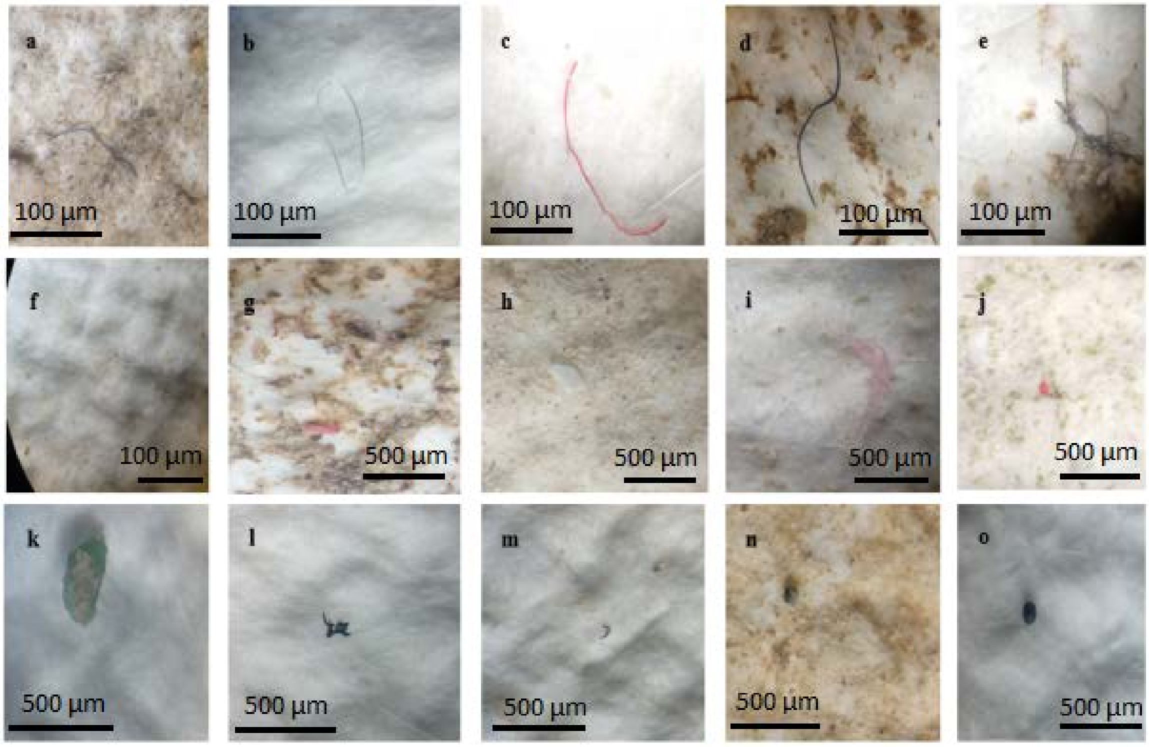

Figure 2. Categories of sampled microplastics (water and sediment of Joumine stream): a-f; fibers, g-k; fragments, l-m; films, n-o; pellets.

and subjected to Fourier transform infrared spectroscopy in attenuated total reflectance mode for analysis. Fourier-transform infrared spectroscopy in attenuated total reflectance mode (FTIR-ATR) was used to identify the sampled plastic polymer. Various particles categories (fibers, fragments, films, and pellets) were subjected to the FTIR analysis. The spectra were given by PerkinElmer Spectrum Two FTIR Spectrometer with a DTGS detector. The analyses were carried out at the sample surface. The measurement resolution was set at 4 cm^{-1} with 32 scans. The plastic polymers were characterized based on the revealed absorption bands in accordance with the literature (Toumi et al., 2019).

2.4. Statistical analysis

Data concerning abundance, size, shape, and color were analyzed and represented through EXCEL (version 2013, Microsoft) and IBM SPSS® software (version 25, IBM Corp.). PMs found in sediment were denoted as particles/50g dry weight, while those found in water were denoted as particles/L. IBM SPSS® software (version 25, IBM Corp.) was utilized for statistical analyses. When ANOVA detected notable differences, post hoc comparisons were carried out using the Tukey HSD test (THSD), with statistical significance set at $p>0.05$.

3. RESULTS

Four microplastics that were present in the blank samples were excluded from the obtained data. MPs were detected in all five sampling sites, but the types and quantities of MPs varied greatly between sites, both in the water matrix and sediment. Figure 2 shows microplastic categories isolated from water and sediment samples and then observed under a stereomicroscope.

3.1. Analysis of microplastics in water

The average MPs concentration in the water of the five sites was approximately 8.87±3.94 particles per liter. The highest concentration of MPs was detected in the El Arima site (14 particles/L), while the Souidia site had the lowest concentration (5 particles/L) (Figure 3a).

Water samples were recorded to have fibers, fragments, and films (Figure 3a). The size of MPs varied based on the type of microplastics found in the water samples, with fiber sizes range from 671 µm to 1028 µm (i.e., 0.671 mm to 1.028 mm), while fragment and film sizes range from 121 µm to 381 µm

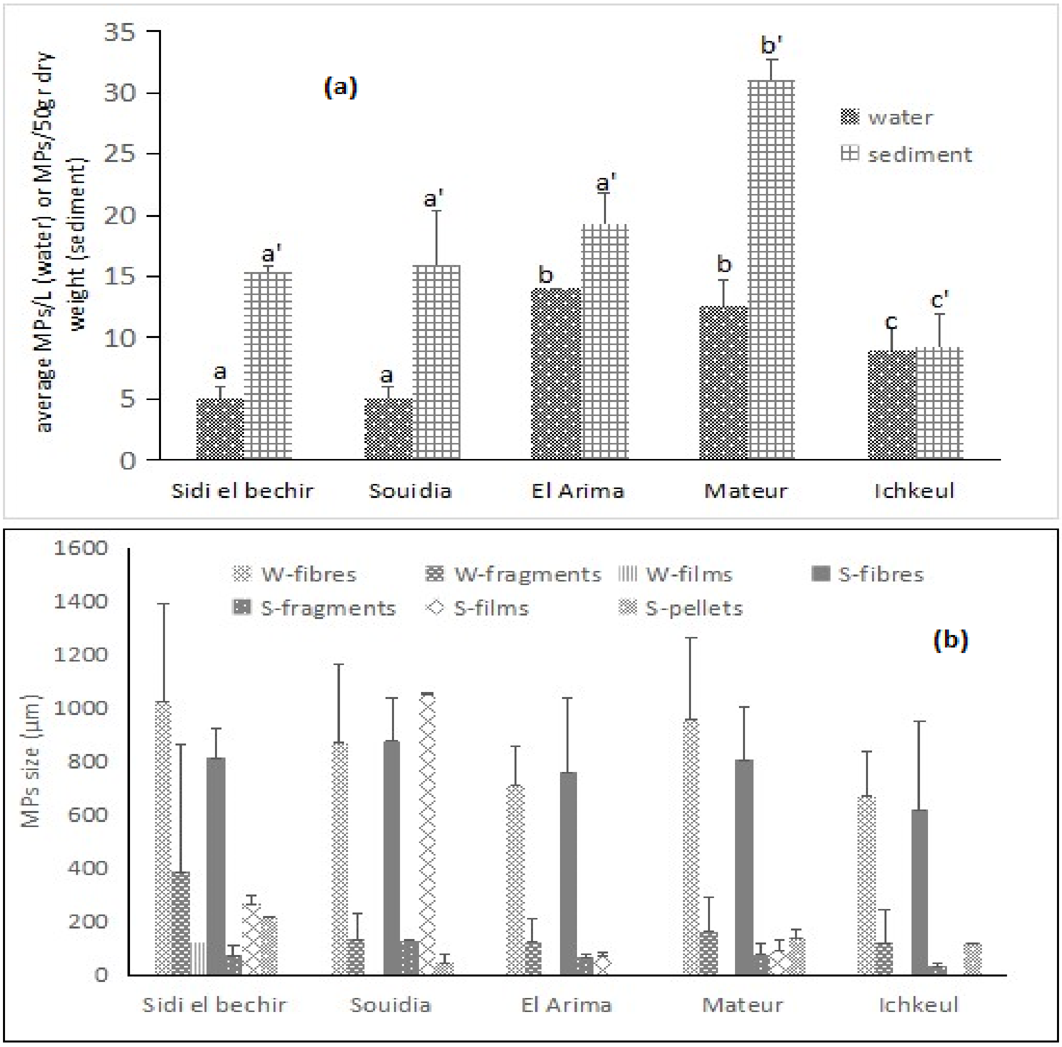

Figure 3. Abundance of microplastic particles (a) and their sizes (b) in waters and sediments of sampling sites. ($p<0.05$: significant difference (ANOVA, Tukey's HSD)). W: water and S: sediment.

(i.e., 0.121 mm to 0.381 mm) (Figure 3b). Fibers had the highest frequency, accounting for 72.1%, while fragments and films made up 27.12% and 0.78% of the total samples, respectively (Figure 4a). In terms of color distribution, black had the highest percentage at 27%, followed by red at 23%, blue at 22%, white at 17%, green at 8%, yellow at 2%, and clear at 1% (Figure 4c).

3.2. Microplastics in sediments

The average concentration of MPs in the sediment samples across the five sites was 18.20±8.27 particles per 50 g dry weight. The highest concentration was observed in the Mateur site (32 particles per 50 g dry weight), while the lowest was observed in the Ichkeul site (9.33 particles per 50 g dry weight) (Figure 3a). The sizes of MPs varied depending on the type of microplastics present in the sediment samples, with fiber sizes ranging from 620 µm to 879 µm and fragment, film, and pellet sizes ranging from 48 µm to 267 µm, except for a single film in the Souidia site, which measured 1056 µm (Figure 3b). In the sediment samples, four forms of microplastics were identified: fibers, fragments, films, and pellets, representing 34.07%, 2.2%, and 2.57% of the elements present in the surveyed sites (Figure 4b). Yellow was absent in the sediment samples, with white being the dominant color at 39%. Other colors were distributed as follows: black at 21%, green at 18%, blue at 11%, red at 10%, and clear at 1% (Figure 4d). Fibers come from a variety of sources, including household laundry, textiles, and fishing gear, and their small size and buoyancy make them more easily dispersed and deposited in aquatic environments. The other types of MPs, particularly those resulting from industrial activities, are less abundant due to the absence of these sources in the surrounding area. Urban land cover is also closely correlated with microplastic abundance, possibly due to factors such as inadequate waste management strategies and littering.

3.3. FTIR analysis

The comparison of the spectra corresponding to sampled particles was performed by identifying the peaks in the Fourier Transform Infrared (FTIR) spectroscopy analysis and comparing them to the standard spectra of pure or weathered samples. The analysis revealed the presence of polyethylene (PE) in the water sample and phenol-formaldehyde (PF) resins in the sediment sample (Figure 5).

4. DISCUSSION

Our study findings indicate that the concentration of MPs in sediment is significantly higher (~2 times) than in water along the Joumine stream. This is likely due to the crucial role of the sedimentation process in the accumulation of MPs (Li et al., 2019). However, our data cannot be directly compared to those of Scherer et al. (2020) in Elbe River sediments reported much higher concentrations

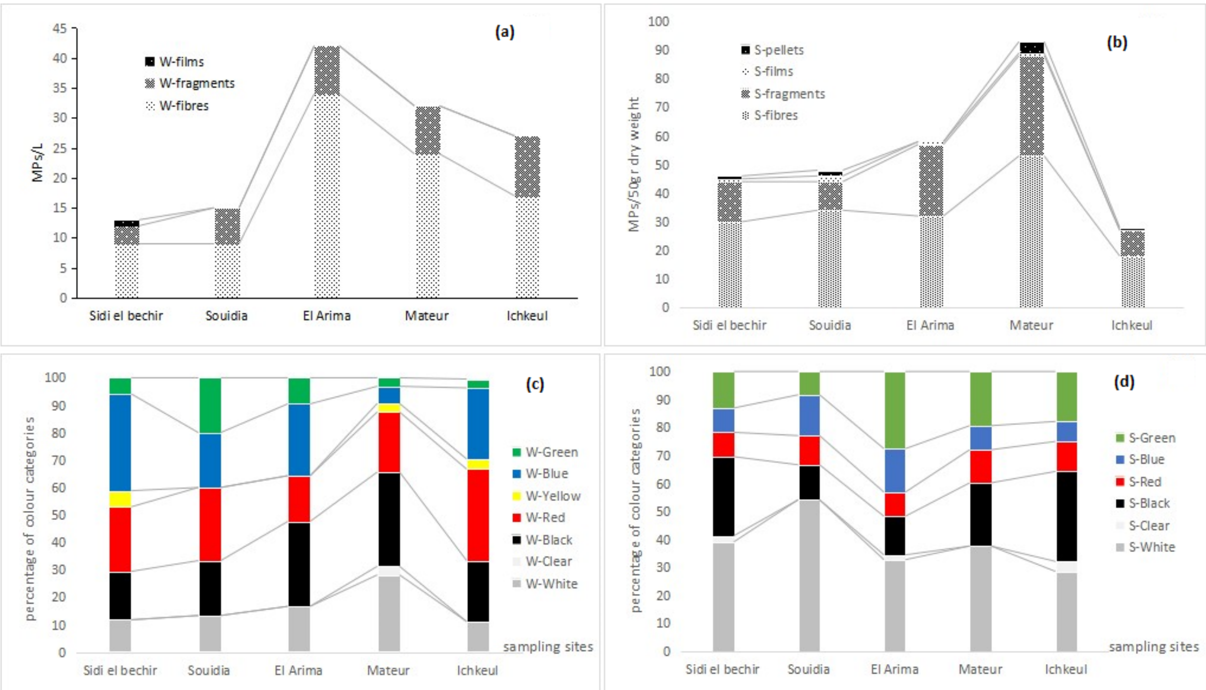

Figure 4. Forms and colors of microplastic particles respectively in water (a, c) and sediment (b, d) of sampling sites.

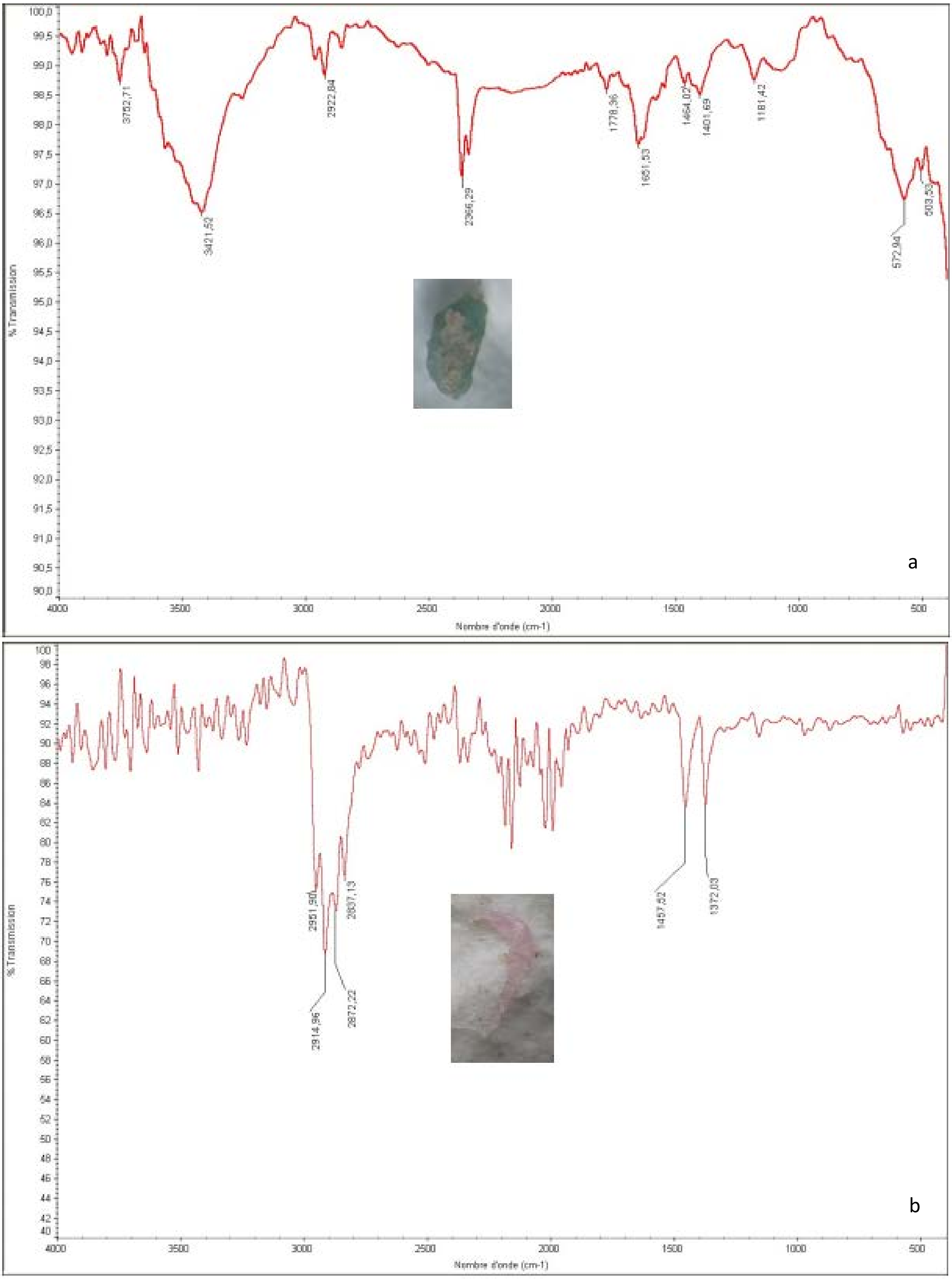

Figure 5. FTIR transmission spectra of polyethylene (a) and phenol-formaldehyde resins (b).

ranging from 2.26×104 to 2.27 × 107 pm^3, representing an average 600,000-fold increase compared to the water phase (0.88 to 12.24 pm^{-3}). However, the characteristics of sediments have a significant impact on the distribution of microplastics. Fine sediments, such as clay and silt, have a high specific surface area, which increases their ability to trap small MPs through adsorption and aggregation (Horton et al., 2017). This structure favors PM accumulation, particularly fibers and fragments, which are easily trapped in fine sediment matrices (Peng et al., 2017). Conversely, coarse sediments such as sand and gravel, typically found in high-energy environments with turbulent flow, are less able to trap fine particles but can selectively retain larger MPs (Van Cauwenberghe et al., 2013; Li et al., 2018). This distribution pattern is critical for understanding the environmental impact of MPs, especially in ecosystems with different sediment types along different flow conditions. In addition, further studies are needed to assess the extent of microplastic degradation in the Joumine stream, taking into account key environmental variables such as UV exposure, microbial activity and physical abrasion.

The amount of microplastics present in water (Table 1) is less compared to the level found in the Saigon River in Vietnam, which was measured at 172-519 n/L according to Lahens et al. (2018). However, the amount found in the Weihe River (3.67-10.7 n/L) as reported by Ding et al., (2019) confirms our findings.

On the other hand, the value we obtained is higher than that reported in the Antuã River in Portugal (0.058-1.265 n/L) by Rodrigues et al., (2018). Our findings on microplastic levels in sediment differ from those of Wagner et al., (2014), who reported a mean MPs value of 34-64 elements/kg in freshwater ecosystems (Rhine River, Germany). Their recorded values were significantly lower than ours. On the other hand, the studies of Horton et al., (2017), Toumi et al., (2019), and Abidli et al., (2018) reported much higher average values of MPs in sediments compared to our findings. Specifically, Horton et al., (2017) found 660 elements/kg in the Thames River, Toumi et al., (2019) reported 4000 elements/kg by dry weight in the Tinja channel, and Abidli et al. (2017, 2018) reported 3000-18000 elements/kg dry sediment in the channel of the Bizerte Lagoon Complex. The water samples from the "Al Arima" site, situated downstream of the Joumine dam, exhibit a notable contrast with those from the "Sidi el Bechir" and "Souidia" sites upstream of the dam. This disparity suggests that the Joumine dam plays a constructive role in detaining microplastics. Although the primary cause of microplastics in the Joumine dam remains unspecified, Turhan (2021) identified wastewater discharge and atmospheric pollution as the main sources of microplastics in the Sürgü dam in Turkey. The presence of microplastic pollution in the Joumine stream is strongly linked to the establishment of human settlements. In the sediment samples, the levels of microplastics detected at the Mateur site, located in proximity to the city, differ considerably from those at other sites. This outcome aligns with the findings of Talbot & Chang (2022), who reported a positive correlation between microplastic concentrations, population density, and the degree of urban development. Our study identified four types of MPs, namely fibers, fragments, films, and pellets. The high proportion of fibers and fragments suggests that secondary MPs resulting from the breakdown of larger plastic debris are more prevalent than primary MPs (Zhao et al., 2016).

In our investigation, microfibers emerged as the most abundant form of MPs in both water (72.1%) and sediment (61.16%). Similarly, Forrest et al., (2019) found that fibers accounted for 98% of the MPs in the Ottawa River, Canada, while Wu et al., (2020) reported that 94% of the MPs in the Yangtze River estuary sediments were fibers. Synthetic fibers

Table 1. Microplastic concentrations in sediment and water samples from the Joumine stream and other selected streams from the literature

Location	Level in Water	Level in Sediment	Types of MPs	Size Range	References
Joumine Stream, Northern Tunisia	8.87 ± 3.94 particles/L	18.2 ± 8.27 particles/50g dry weight	Fibers, Fragments, Films, Pellets	0.24 - 1.45 mm	This Study
Thames River, United Kingdom	0.88 - 12.24 particles/m³	34 - 64 elements/kg	Fibers, Fragments	Not specified	Horton et al. (2017).
Changjiang Estuary, China	4132 ± 2460 particles/m³	Not specified	Fragments, Films, Fibers	0.45 - 5 mm	Peng et al. (2017).
Deep Sea, North Sea	Not specified	4.2 - 12 particles/50g dry weight	Fragments, Pellets	0.3 - 5 mm	Van Cauwenberghe et al. (2013).
Sandy Beaches, Brazil	Not specified	2 - 20 particles/m²	Pellets, Fragments, Films	1 - 5 mm	Turra et al. (2014).
Freshwater Systems, China	3.67 - 10.7 particles/L	72.2 - 380 particles/kg	Fragments, Fibers	0.1 - 4.75 mm	Li et al. (2018).

may arise from different sources, such as wastewater discharges from domestic sewage treatment plants (resulting from the washing of synthetic clothing), emissions from clothes dryers, decomposition of discarded nets and ropes, and cigarette butts (De Falco et al., 2019; Kapp & Miller, 2020; Dris et al., 2018; De Villiers, 2019). Due to their high length-to-diameter ratio, microfibers are easily ingested by aquatic organisms (Dris et al., 2018).

Large plastic items like bottles and rugged plastics mainly decompose into fragments, according to Free et al., (2014). Lin et al., (2018) found a high proportion of fragments in the sediments of the Pearl River, and recent studies suggest that tire wear particles contribute to PM contamination of aquatic environments (Ziajahromi et al., 2020). Black fragments from tire wear particles accounted for 17% of the total MPs in the Charleston Harbor Estuary, USA (Leads & Weinstein, 2019). Film-like microplastics come from weathering and cracking of plastic products like packaging bags, agricultural film waste, and plastic films and enter the natural environment through external forces (Wang et al., 2022). Pellets come from personal cleaning products, cosmetics, or industrial pre-production granules (Hartmann et al., 2019). The presence of seven colors suggests that MPs may have various sources (Munari et al., 2017). Blettler et al., (2017) documented nine colors of particulate matter in the floodplain of the Paraná River, including clear plastic items that could be from plastic bags or bottles, or discolored due to weathering (Wong et al., 2020). Blue plastics are commonly used in synthetic clothing worldwide, as well as in mussel farming (Gago et al., 2018; Digka et al., 2018). However, plastic polymers can be affected by sample processing, such as becoming transparent or discolored when treated with 30% hydrogen peroxide (Nuelle et al., 2014). Furthermore, color perception can be subjective and influenced by various factors, including microscopy quality, illumination and background, changes in the environment over time, and personal factors such as color blindness (Lu et al., 2021). Our study found that fibers larger than 500µm were present in all of the sites we investigated, whereas fragments, films, and pellets were less than 500µm. In contrast, Hu et al. (2018) observed that particles smaller than 500µm were the most common in water and sediment samples from the Yangtze River Delta in China, and the frequency of particles decreased as size increased.

In some samples, the proportion of MPs varied greatly, ranging from 25% to 2%, resulting in potential contamination and causing a lack of peaks or unexpected noise in the FTIR spectra, which can affect the performance of FTIR identification of microplastics (Li et al., 2022). The presence of polyethylene (PE) in water samples can reflect the widespread use of this polymer in packaging, personal care products, and containers, as it is a polymer with high global production (Gada, 2024). Several studies have reported that the main types of MPs in water samples were polyethylene (PE) (20.9%), polyethylene terephthalate (PET) (19.3%), and polypropylene (PP) (18.1%) (Lu et al., 2021). PE polymers are more likely to be present in water samples due to their density and susceptibility to flotation (Sun et al., 2022). Identifying formaldehyde-based resins such as PF, MF, and CSF can be challenging due to their rigid structure, which makes it difficult to obtain high-quality spectra, and the lack of reference materials for these polymers. In addition, PF spectra are generally of lower quality due to the dark green color of the resin, which can make it challenging to obtain a good spectrum (Bell et al., 2019). PF phenolic resin, which is made from non-plant materials, was the first plastic used for a wide range of bearing shapes, types, rings, and cages.

5. CONCLUSION

This study provides a longitudinal analysis of the levels of microplastics (MPs) enrichment in the Joumine stream, a tributary of the Ichkeul preserved wetland, by determining their abundance, shape, size, and type. The impact of the dam on microplastic dynamics is highlighted by the highest concentration of MPs in water samples from the Al Arima site, and the highest concentration of MPs in sediments is found in the Mateur site. The investigated ecosystem is surrounded by forest upstream and locally rural areas. The presence of the dam seems to modify water velocity and various particle dispersion. The downstream is bordered by urban, industrial and agricultural areas from which they receive solid and wastewater discharges. A treatment plant is located close to Mateur City, thus impacting the lowland stream part as revealed by the recorded data for the ending sites. The pollution of the Joumine stream by MPs is strongly linked to anthropogenic pressure, even though the stream is mostly situated in a rural area with low anthropogenic activity. The contamination level is average compared to existing literature, underscoring the need for preventive measures to protect our water resources and biodiversity.

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Declaration of interest

The authors do not have any potential conflict of interest.

Data availability statement

The corresponding author can provide the supporting data for the conclusions of this study upon a reasonable request.

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