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Research Article

Overview of microplastics abundance in the surface waters of Ghar el Melh and Kalâat el Andalous, Gulf of Tunis (Tunisia, Mediterranean Sea)

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Abstract: Microplastics have been detected in various aquatic environments, such as surface seawater, sediments and aquatic organisms, with specific sampling strategies required for each environment. As part of the scientific work of the Plastic Busters CAP project, a sampling campaign was conducted in the Gulf of Tunis in September 2023, targeting two transects located in Ghar el Melh (T_1) and Kalâat el Andalous (T_2), each comprising three sampling stations. Results revealed that microplastics concentrations in both sampling area varied significantly from 20325.2032 to 104336.043 particles/km^2. The highest concentration was located at the nearest station to the shoreline T_1-S_1, which remain a strong influence of land-based sources. The most ubiquitous microplastics particles size ranged from 1 to 2.5 mm, indicating a predominance of small-sized debris likely resulting from the fragmentation of larger plastic items. The evaluation of the morphological classification showed a high dominance of film-type and white color microplastics. Polymer data in this study were obtained through spectral characterization using Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance (FTIR-ATR) mode, revealing that high-density polyethylene (HDPE) was the most abundant polymer in both transects.

Keywords: Sea pollution; Plastic debris; Pollutants; Surface waters; Water sampling; Spectral analysis; Tunisia; Mediterranean sea.

1. Introduction

Plastic pollution are continuously discharged into marine environments on a daily basis, from the shallow coastal areas to the deepest areas sampled, as well as remote and sensitive places on earth, reflecting the uniform global distribution of this pollution (Ford et al., 2022).

By referring to the Guide of policy makers in Tunisia “Stop the plastic flood” published by WWF in 2019, it can be stated that approximately 8.5 kilotons of plastic waste find their way into the Mediterranean Sea annually. Furthermore, research carried out by (Lebreton et al., 2012) estimates that the concentration of microplastics in the Mediterranean will increase by 8% over the next 30 years. According to (OECD, 2023) the amount of plastic accumulated in rivers and oceans is projected to increase from 152 million tons (Mt) in 2020 to 226 Mt by 2040. The research findings revealed that, out of 141 surface marine water samples collected from various oceans, 88% contained microplastics (Cózar et al., 2014). In Tunisia, 0.08 million tons of plastic waste ends up in nature every year due to poor waste management. This plastic pollution affects directly the tourism, maritime transport and fishing economies, causes the Tunisian economy to lose around 20 million dollars each year (Dalberg Advisors et al., 2019). Furthermore, the complex interaction between plastic pollution and climate change suggests that the real impact of plastics on the economy and environment is likely higher than current estimates suggest. This interaction represents an additional stressor for marine ecosystems. Due to their persistence and accumulation in various environmental matrices such as sediments, the water column, soil, the cryosphere, and the atmosphere (GRID-A Arendal, 2024), these micropollutants are considered emerging pollutants. They can remain stable in aquatic environments for thousands of years owing to their chemical stability, large specific surface area, and strong hydrophobic nature (Lohmann & Belkin., 2014).

Plastics are classified into different categories depending on particle size, including macroplastics (>25 mm), mesoplastics (5 to 15 mm), microplastics (<5 mm) and nanoplastics (<100 nm) (Bodzek & Pohl., 2022).

Microplastics are further classified into two categories. Primary microplastics are intentionally manufactured in microscopic dimensions for industrial and domestic purposes. Designed to be microscopic such as microbeads, they are used either directly (e.g., in cosmetics) or indirectly (e.g., as pre-production pellets) and secondary microplastics which result from the fragmentation of large plastic waste (by physicochemical degradation) into particles smaller than 5 mm (Alvarez et al., 2020). These discarded plastic wastes were considered as a transport medium for various toxic chemicals, which attach to many plastic particles and are very harmful to all aquatic organisms. Their persistence in the ocean has recently been demonstrated as well as their capacity to adsorb certain persistent hydrophobic chemical contaminants present in the marine environment, notably toxic persistent organic pollutants (POPs: PAHs, PCBs, etc.) and heavy metals (Fe, Mn, Al, Pb, etc.) (Ghiglione et al., 2023).

They eventually become transmitted to humans through the food chain, causing significant health problems (Shen et al., 2022). These particles can be composites or copolymers (mixtures of different polymers) (Hale et al., 2021), they adsorb more easily the organic chemical pollutants. According to (Alvarez et al., 2020) Low-density polyethylene (LDPE) has the capacity to adsorb various organic pollutants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBDEs). Similarly, polypropylene (PP) and polystyrene (PS) tend to adsorb PAHs, hexachlorocyclohexanes (HCHs), and chlorinated benzenes (CBs). In addition, polyvinyl chloride (PVC) can sorb compounds such as phenanthrene and dichlorodiphenyltrichloroethane (DDT). Furthermore, polyethylene pellets have been shown to adsorb several trace metals, including Fe, Al, Mn, Pb, Cu, Zn, and Ag. In addition, a study carried out by (Chaabane, 2019) confirms that chemical and microbiological pollution resulting from plastic waste constitutes a major environmental problem affecting the coastal systems. For this, further research should focus on designing and improving the identification technique as well as the processing of the sampled polymer according to its type, its shape as well as the nature of the additives detected on its surface, while taking into account its relative risks affecting physical behavior and toxicological results, at the ecosystem level (Hale et al., 2021). Microplastic pollution has emerged as a major environmental concern due to its persistence in ecosystems, its potential for bioaccumulation, and its detrimental effects on both aquatic organisms and human health (Chouchene et al., 2023). It is imperative to understand the sources and impacts of this pollution on the environment, resulting from the gradual increase of microplastics in the aquatic environment.

This study investigates the presence, abundance, and characteristics of microplastics in surface waters at two distinct sites in Tunisia: Ghar el Melh and Kalaât el Andalous. The primary objective of this research is to quantify the concentration and identify the types of microplastics present at these sites, as well as to assess their potential sources of contamination.

2. Materials and Methods

2.1. Study site & Sampling

The Gulf of Tunis was considered as a pilot area for the Plastic Busters CAP project (**Figure 1**). The selected sampling sites were chosen based on their potential to represent areas impacted by marine plastic pollution. Sampling was carried out at the end of the summer season, in mid-September 2023. In total, six stations were sampled (**Table 1**). In each Transect, three stations located respectively 1.5, 3 and 6 miles from the coast. The first transect chosen was (T_1, Ghar el Melh) situated on the Mediterranean coast, approximately 60 kilometers northeast of the capital city, Tunis. Ghar el Melh beach is located between the new Boughaz pass and the old mouth of Oued Medjerda. It is made up of a dune cord (called lido) which separates the Ghar el Melh lagoon from the sea (Hzami, 2022).

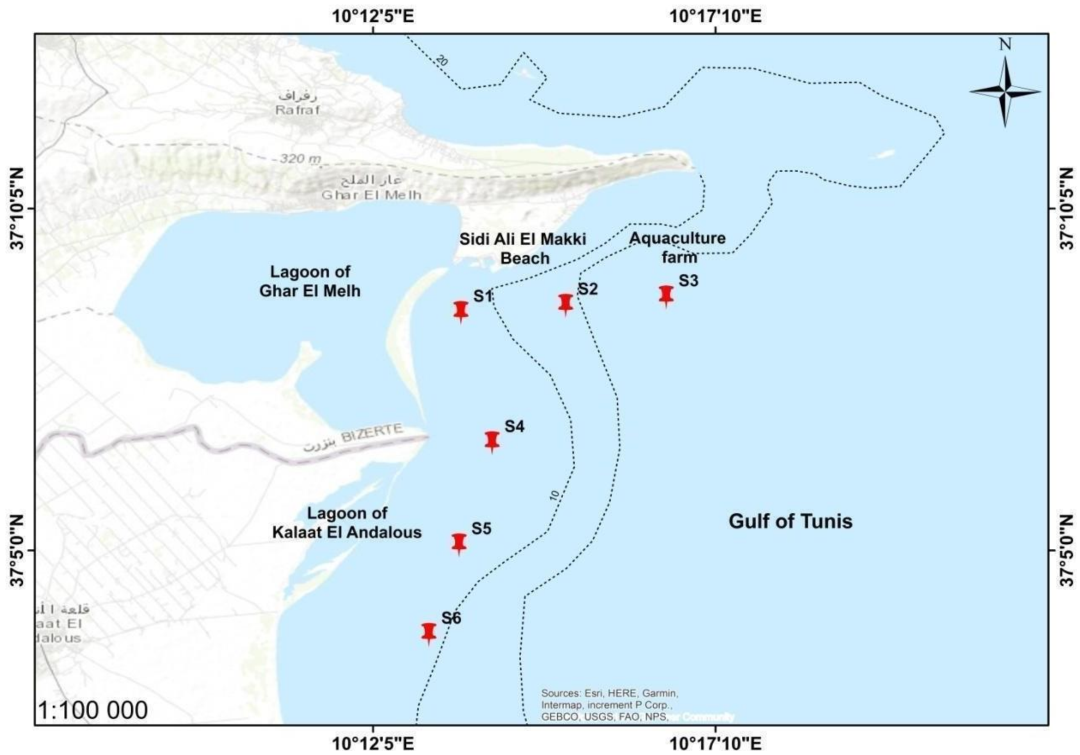


Figure 1: Study area and sampling station.

Table 1 - Sampling Station

Transect	Station	Latitude	Longitude
T_1: Ghar El Melh	S_1	37° 8'31.70"N	10° 13'23.39"E
	S_2	37° 8'38.16"N	10° 14'56.68"E
	S_3	37° 8'45.54"N	10°16'26.16" E
T_2: Kalaat El Andalous	S_4	37°6'35.56" N	10°13'51.21" E
	S_5	37°5'4. 41" N	10°13'21.73" E
	S_6	37°3'44.52" N	10°12'54.85" E

The lagoon and surrounding areas provide a picturesque setting, attracting visitors for both cultural and natural attractions. This area is distinguished by intense tourist activity, fishing, agriculture, aquaculture and urbanization, which indicate a growing threat of marine debris along the coast; this is what prompts us to continue our work and focusing our efforts on this pilot area. Ghar el Melh was recognized as a wetland of international importance and, in 2018, became the first city in North Africa and the Middle East to receive the prestigious Ramsar Wetland City Accreditation (Mzoughi, 2013). This recognition highlights the city's dedicated efforts toward the conservation and maintenance of its wetlands. It is also classified as the Coastal Ecohydrology theme by UNESCO in Africa and the Mediterranean.

The second transect (T_2) overlooks the picturesque lagoon of Kalaât el Andalous, a residential district situated within the Ariana Governorate of Tunisia. Kalaât el Andalous lagoon is located in the center of the western bay of the Gulf of Tunis, between the geographic coordinates 37°05'22"N to the north and 10°11'49'E to the south. This lagoon is located in the deltaic plain of Oued Medjerda, bordered by the ancient mouth of the Oued to the north and Sebkha Sidi Bahroun to the south.

Kalaât el Andalous lagoon is in an elongated elliptical shape with a surface area of 163 hectares, its length is 5.5 km, its width is 0.7 km, its perimeter is 8.4 km and its average depth is 2 m. These two sampling sites were selected based on the environmental and anthropogenic factors affecting the Ghar el Melh and Kalaât el Andalous lagoons. These areas are particularly vulnerable to multiple stressors, including port activities that contribute to pollution through fuel leaks and discharges, which alter light penetration and oxygen levels. In addition, increasing population density, urbanization, and industrialization in the surrounding areas introduce significant pollution loads via water discharges, further reducing water transparency. The ecosystem is also impacted by treated wastewater overload and irrigation runoff. These criteria ensure that the sampling sites provide a representative understanding of environmental pressures, including plastic pollution, and their impacts on the lagoon ecosystem. However, recognizing potential biases, such as focusing on highly impacted areas, could improve the transparency of the study (Khsiba et al., 2021).

The selection of these sites was intended to assess microplastic contamination in regions of ecological and economic importance, as microplastic pollution poses potential risks to marine life and human health. Kalaât el Andalous, being closer to urban centers, is influenced by industrial and domestic waste, providing a contrast in the sources of microplastic pollution. These factors make the sites relevant for understanding microplastic pollution from both secondary and primary sources. Both sites have ecological significance, Kalaât el Andalous lagoon was highly valued for their socio-economic importance, which makes them particularly vulnerable to natural threats (such as marine submersion, floods and storms) and to anthropogenic pressures (notably urbanization, wastewater discharges, pollution and coastal developments) (Khsiba et al., 2018); moreover Ghar el Melh has significant local pressure from agricultural runoff, fishing activities, and urban development in nearby areas, connected to the Mediterranean through a dredged opening and receives untreated sewage from two towns and a catchment of 131 km^2 (Kock Rasmussen et al., 2009). By comparing the results found in these two sites, we aim to study the abundance of microplastics in this region characterized by its Mediterranean climate and its bioclimatic positioning between sub-humid and semi-arid zones.

2.2 Application of data resources in an Ethical Manner

The PB CAP project implemented metadata deposition through infrastructure services provided by the National Institute of Marine Science and Technology (INSTM). This work ensures traceability of data sources, incorporating fundamental geographic details (such as date, time, latitude and longitude) at the study area level and methodological information (including monitoring devices, sample processing time, sample volume recovered and storage conditions) throughout the sampling process. This was achieved by assigning unique sample identifiers in the form of barcode labels on the sample container and accompanying data sheet. The digitization and archiving of all information was carried out carefully using various field measurements.

2.3. Methodology for monitoring microlitter on the sea surface using manta net towing: PB CAP toolkit

According to the PB CAP protocol, the microplastic sampling process was meticulously carried out using a specialized manta net with a precisely calibrated mesh size ranging between 300 and 335 µm, complemented by an integrated flow meter. The deployment of the manta trawl occurred at an optimal speed of approximately 2.0 knots, sustained throughout a 20-minute journey between stations. Crucially, both the departure and arrival locations were recorded using GPS, capturing the entire route for comprehensive data collection. This meticulous approach ensures the accuracy and reliability of the collected samples, contributing to the robustness of the overall research methodology (Fossi et al., 2022).

2.4. Analysis of sea surface water samples

The quantification of microplastic abundance can be achieved through different methods, and depends on factors, such as sample matrices, sampling technique, and analysis methods. To ensure the reliability of the analysis and the detection of microplastic pollution in water samples, we have relied on a harmonized standard protocol elaborated by the Plastic Busters CAP researchers. After collecting water samples, each sample was rinsed with seawater over a 300 µm metal sieve and transferred to glass jars, all natural debris removed and rinsed separately on the sieve. Then, a 70% ethanol solution was added to the sampled water, to be preserved and frozen for chemical analysis. Rigorous application of a strict scientific protocol, including methodical cleaning of the equipment, would have reduced the risks of contamination and ensured more reliable and less biased results (Fossi et al., 2022).

2.5. Identification of microplastics in sea surface waters

2.5.1. Morphological characterization

Visual analysis of microplastic particles was carried out using a highly reliable stereomicroscope (ZEISS) to determine the shape, size and colors of each microparticle (**Figure 3**). Each microplastic particle was removed from the filter surface using stainless steel tweezers, preserved and collected in petri dishes for long-term storage. These Petri dishes must be weighed before and after adding the sample studied.

$\Delta_m = m_{Sample} - m_0$.

We should take into consideration the number of particles found in each station, for those which exceed a hundred plastic particles; we limit our spectroscopic analysis to only 10% of the weight of the Petri dishes.

2.5.2. Chemical characterization

After having isolated and detected the potential MPs in each sample, it is necessary to confirm or not the synthetic nature of these particles. This validation step is essential to accurately characterize the contamination. The identification of the polymer type was carried out by Fourier Transform Infrared Spectroscopy-Attenuated Total Reflectance (FTIR-ATR). All microplastic particles were placed on a diamond germanium cell (ATR Golden Gate) **(Figure 6.b)**, where the sample holder was rinsed with ethanol after each chemical analysis of microplastics to ensure cleanliness and avoid cross-contamination. The nature of the detected polymer is determined by comparing the sample's spectrum with reference spectra available in the SPECTRUM software's microplastic database. A polymer is identified when there is a sufficient match between the sample spectrum and a reference spectrum. Indeed, the software executed two preprocessing phases, starting with baseline correction followed by the normalization of the spectra, this procedure employed k-nearest neighbors classification. The training database included a well-defined set of spectra representing microplastics (MPs) and other particles, such as natural organic materials. During the identification process, if all the k-nearest neighbors belonged to the same class, the spectrum was directly identified; otherwise, a majority vote was employed. Upon confirmation of the plastic nature of the particles through FTIR analysis, both the quantity and mass of the microplastics were validated. Based on this information, microplastic concentrations were calculated in terms of quantity (items/km^2) (**Figure 9.a**) (Kedzierski et al., 2022).

2.5.3. The limits resolution of the analytical techniques used

Two widely employed techniques for identifying and quantifying microplastics are stereoscopic microscopy and Fourier-transform infrared spectroscopy (FTIR). While both methods are effective, they exhibit resolution limitations, particularly in detecting microplastics smaller than 10 µm. These constraints can lead to an underestimation of microplastic concentrations, potentially compromising the accuracy and reliability of the data. Calibration and validation of analytical instruments like FTIR-ATR can involve reproducibility tests through repeated measurements on identical samples. This approach can be enhanced by using certified reference materials and evaluating the detection and quantification limits to ensure the instruments reliability in accurately identifying microplastics in water matrices (Jung et al., 2018). In addition, identifying the physical and chemical properties of microplastics and their sources helps identify critical areas where pollution needs to be addressed.

2.6. Methodological Framework

This section provides a comprehensive overview of the core protocols employed in microplastic analysis, as outlined in (**Table 3**). The methodology encompasses the following steps:

- ***Sampling:*** Conducted using a manta net to collect surface water samples.
- ***Pretreatment*:** Aimed at removing organic matter and isolating microplastics from the collected samples.
- ***Separation:*** Performed with a 100 µm metal filter to extract microplastic particles.
- ***Characterization:*** Conducted using a stereomicroscope for visual inspection and preliminary classification.

- ***Identification:*** Finalized through Fourier-transform infrared spectroscopy (FTIR) for precise material identification.

3. Potential risks of detected microplastics to ecosystems and public health

The widespread presence of plastics in the environment poses significant health risks to humans. These materials interact constantly with natural systems and can enter the body through ingestion, inhalation, and direct contact. Among these pathways, oral ingestion is the primary route of exposure. Microplastics are often found in food and beverages, such as seafood, drinking water, and salt, leading to unintentional consumption. Inhalation is also a concern, particularly in urban areas where airborne microplastics are prevalent, while dermal contact through products like cosmetics and textiles provides another exposure route. Furthermore, the ability of microplastics to migrate between air, water, and soil exacerbates their impact; this mobility increases the likelihood of simultaneous exposure through multiple pathways, amplifying health risks (Li et al., 2023). This research aims to identify the different types of microplastics in order to better understand exposure pathways, determine sources of bioaccumulation and analyze microplastic contamination in the environment as well as on human health.

4. Results

4.1. Physical properties (Size, shape, and color) of microplastic

All potential particle items were manually sorted out from the sample and categorized by size, color, and shape (fragment, filament, pellet, film, microbead and foam). The main forms observed in the water samples were films 58%, filament 21%, fragments 17%, pellets 3%, and microbeads 1%. The results found are mentioned in (**Figure 2** and **Figure 3**).

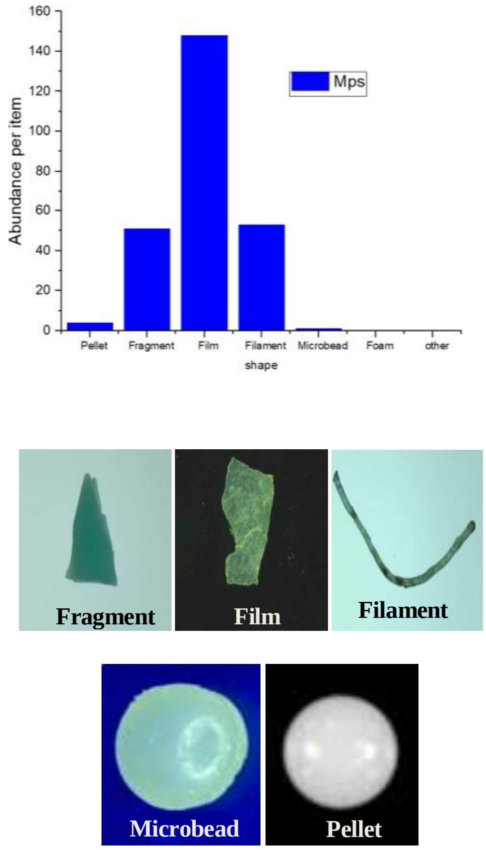


Figure 2. Predominance of the different shapes of Microplastics isolated from water samples.

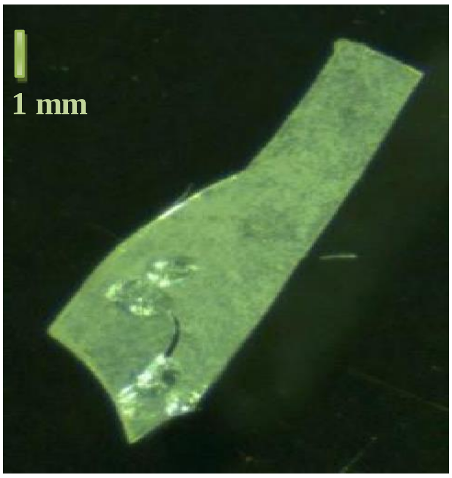


Figure 3. Microscopic observations of the most abundant shape (Film) from station S_1.

All identified particles were classified according to their size, ranging from 0.3 to 5 mm.

The large micro-litter (LML) category, encompassing particles from 1 to 5 mm, along with large small debris, represented the most abundant microplastic type, accounting for 44% and 47% of the total in the two transects analyzed $T_1(S_1, S_2, S_3)$ and $T_2(S_4, S_5, S_6)$ (**Figure 4**). This finding indicates a higher prevalence of larger microplastic fragments in the sampled areas. In contrast, smaller particles in the 0.5 to 1 mm size range were considerably less abundant, comprising only 9% of the total microplastics in the two transects studied.

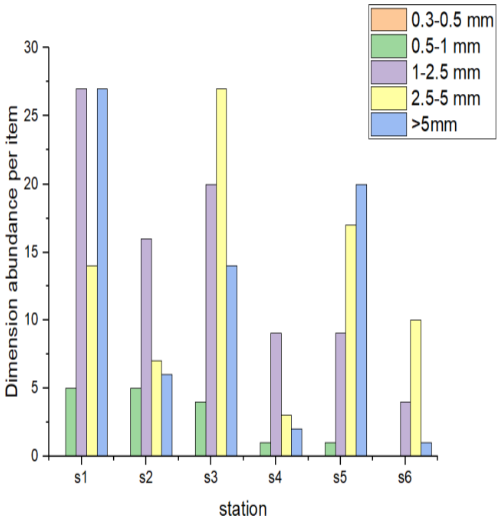


Figure 4. Predominance of the different size classes of Microplastics isolated from water samples.

Furthermore, the color analysis of the microplastics, illustrated in (**Figure 5**), further substantiates these findings, showing that opaque white particles were the most prevalent, constituting 39% of the identified microplastics. This was followed by transparent white particles, which also represented a significant portion of the total. In comparison, microplastics of other colors, such as blue, red, black, green, and miscellaneous shades, were observed in much lower percentages.

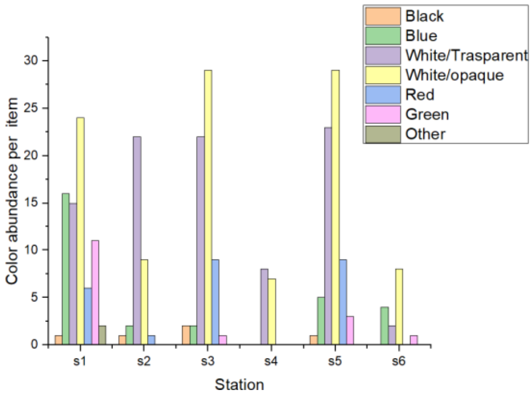


Figure 5. Predominance of the different colors of Microplastics isolated from water samples.

4.2. Analysis of the chemical composition of microplastic particles

The IR spectroscopic study was carried out by irradiating a sample with an infrared beam subjected to molecular vibrations, absorbing a portion of the incident energy. The infrared spectrum (**Figure 7**) reveals absorption bands with frequencies characteristic of the chemical nature of the compound. It allows the detection of characteristic vibrations of chemical bonds and facilitates qualitative and quantitative analysis of the chemical functionalities within the polymer (Boudrahem et al., 2014). Chemical analysis is a necessary step to validate reliable identification of the detected compounds.

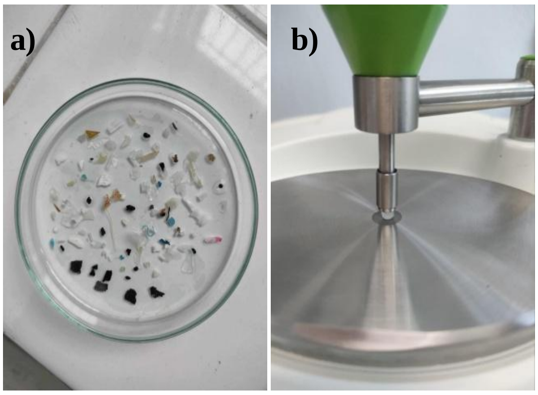


Figure 6. (a) Microplastic particule collected from station S_1, (b) Microplastic particle on ATR Diamond Cell.

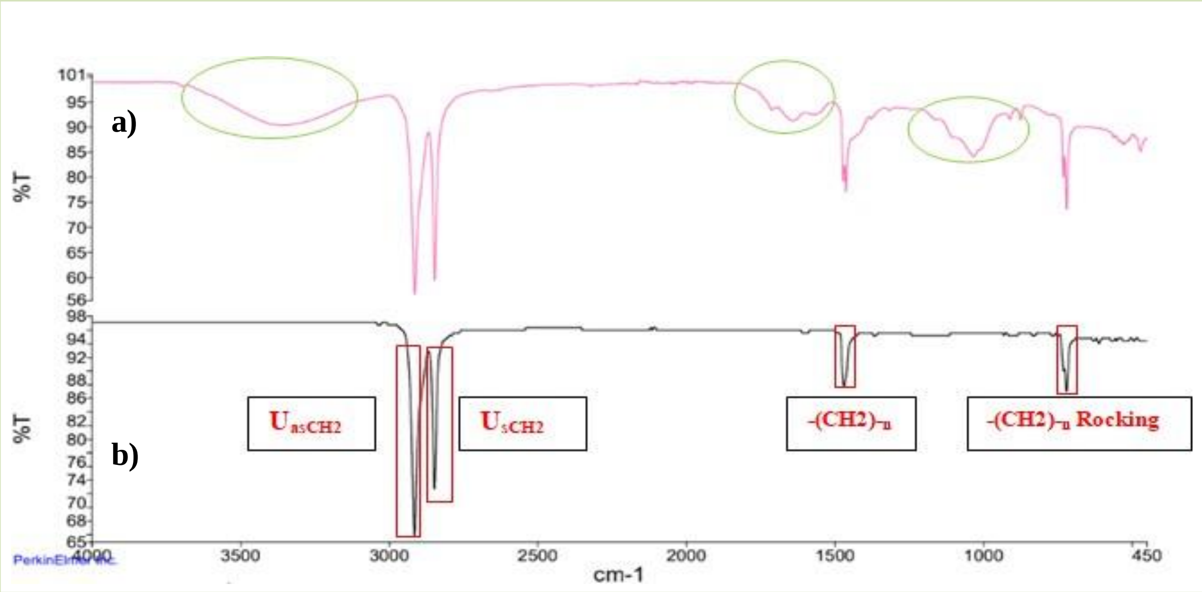


Figure 7. HDPE Spectra: (a) The sample analyzed spectrum ; (b) The reference spectrum of HDPE

The chemical characterization was carried out by Fourier transform spectroscopy to determine the nature of the polymers detected (**Figure 6**). A total of eight distinct polymer types were identified (Nylon, Polyethylene (PE), Polypropylene (PP), Polyester, High density polyethylene (HDPE), Polyamide, Polyvinyl and Polystyrene) (**Figure 8**). The most reported type of polymers was high density polyethylene; they are widely detected in aquatic environments. Their prevalence is associated with their wide use in packaging materials, storage containers, food packaging, water bottles, plastic bags and fishing nets (Ramaremisa et al., 2022).

Our spectroscopic database enables us to conduct a specific analysis for each selected sample. For this purpose, a spectral analysis was performed on each collected particle. Here, we present one of the most dominant particles: an opaque white HDPE film, collected at station S1 (**Figure 3**). The corresponding spectrum (**Figure 7**), obtained from this specific particle, shows an accessible spectral range from 4000 cm^{-1} to 450 cm^{-1}, in mid-IR (MIR) mode, it is the most informative part of the spectrum, as it contains a well-resolved plethora peaks that highlight the analyzed microplastic sample.

This part of spectrum indicates a thin intense band at 2925 cm^{-1} attributed to **U_{asCH2}** which reflects an asymmetric stretching vibration, as well as a less intense band which resonates around 2850 cm^{-1} indicates a symmetric stretching vibration **U_{sCH2}**. In addition to a representative band of deformation vibration in-plane (shear) **$-(CH_2)-_n$** appears around 1470 cm^{-1} and an intense band resonates around 720 cm^{-1} attributed to an out-of-plane deformation vibration (rocking) which only appears if there are more than four CH_2 in a row, this is the case of monomer chain (polymer). By comparing the spectra of the reference HDPE (in black) and the HDPE defined during the analysis (in pink), we notice a modification at the molecular chains level which in agreement with other research (Da Costa et al., 2018) including the evaluation of the degradation of polyethylene in seawater. This modification was characterized by the presence of an intense wide band which resonates around

3300 cm^{-1} which gives rise to an OH functional group. Thus presents a superposition of several peaks, resulting in a valence vibration of the hydroxyl group and which can be explained by the removal of hydrogen H_2 by free radicals during a thermal oxidation of HDPE, either before sampling at sea level (photodegradation) or when adding an ethanol solution to the sample taken in order to preserve it before processing. The bands which resonate towards the 1050 cm^{-1} and 1600 cm^{-1} regions correspond respectively to the C-O ether group and the C=O carboxyl group, which bring together different species strongly linked to the polymerization conditions which can refer to excipients linked to contamination of the surface of the plastic film or the evolution of the photodegradation mechanism which manifests itself by the appearance of chemicals (Fanon et al., 2019).

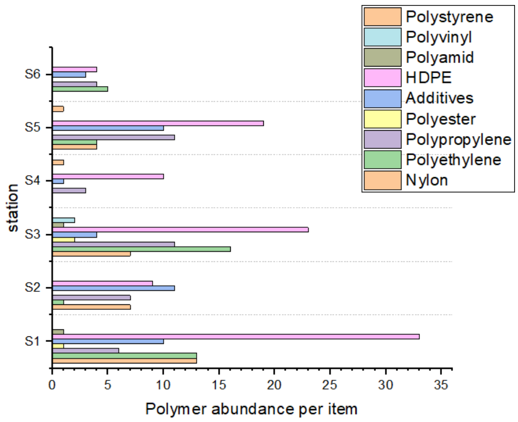


Figure 8. Microplastic particles identified in water samples collected from the Gulf of Tunis.

4.3. Study of the abundance of microplastics in surface waters

The number of MPs found in water samples was expressed as items/km^2, where the area covered (km^2) was calculated by multiplying the width of the mouth of the net by the distance covered (km) during the tow.

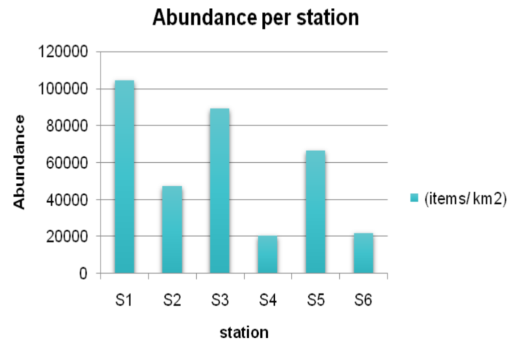
$$Abundance = \frac{nbr\ of\ MPs}{D * Net\ length}$$

Microplastic abundance varies by location, the highest abundance was observed at three stations (S_1, S_3 and S_5). Each surface water sample reflects specific criteria related to its environment. The sample S_1 was collected in the immediate vicinity of the coast of Ghar el Melh, indicates the highest level of abundance of microplastics; this high concentration can be attributed to direct inputs related to human activity and coastal currents. The station S_3 indicates a high abundance of microplastics due to the presence of aquaculture cages, these tributaries carry large quantities of marine debris, constitute a significant source of microplastics. Station S_5, meanwhile, is close to the former outlet of Oued Medjerda and facing the Kalaât el Andalous lagoon. The state of the lagoon's waters is threatened by the increase in population density, the high rate of urbanization and the industrialization of surrounding towns (Khsiba et al., 2021) makes this area particularly vulnerable. The results obtained during the last campaign, conducted in September 2023, are summarized in (**Figure 9**) and (**Table 2**).

Table 2. Abundance of microplastics (items/km^2) found in the samples collected along the two transects (T_1 and T_2) during the Autumn Campaign 2023.

Transect	Abundance (items/ km^2)	Stations	Abundance (items/km^2)
T_1	241192.41196	S_1	104336.0434
		S_2	47425.47425
		S_3	89430.89431
T_2	108401.08401	S_4	20325.20325
		S_5	66395.66396
		S_6	21680.2168

a)



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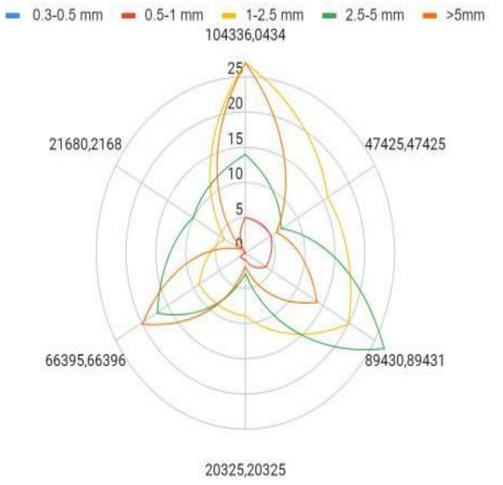

Figure 9. Comparison of the distribution of microplastics according to their abundance in water samples: (a) abundance per station, (b) abundance per size classes.

5. Discussion

Several researches have attempted to delineate the magnitude of microplastic contamination in ocean waters, globally, within the Mediterranean region, and specifically in Tunisia. Table 3 shows the different sampling techniques applied from 2014 to 2024, where the majority of research mentioned the use of a manta net to collect microplastic particles. A common feature in most research efforts is the use of stereomicroscopy for the visual identification and quantification of plastic particles coupled with chemical identification using the FTIR spectrometer (**Figure 6.b**). This spectroscopic method had a dual purpose, firstly to accentuate significant alterations in the molecular structure of the polymers, and secondly, to monitor the oxidation kinetics of the identified particles (Hettal, 2022).

5.1. Correlation between microplastic properties and pollution sources for effective mitigation

The microplastics detected in this study (**Figure 10**), were mainly in the form of films, followed by filaments and fragments. These forms are generally associated with multiple sources of microplastics, but are mainly categorized into land-based and ocean-based sources. The land-based sources contribute 80-90% of microplastics in water bodies, which include plastic bags, plastic bottles, personal care products, plastic incinerators, construction materials, and textiles. Similarly, the fragmentation of larger plastics (such as plastic bags, fishing nets, and plastic waste) also accounts for 10-20% of microplastic releases into water bodies, particularly from ships, plastic waste on beaches, and fishing gear (Osman et al., 2023). In the ocean, sizable debris presents a threat to safe navigation. Every year, thousands of containers are lost from ships, creating a serious risk of collision as they float for weeks or even months before sinking. In addition, blockages in water pipes or waste entangled in ship propellers cause hundreds of incidents or accidents each year, further compounding the economic and safety problems associated with marine litter (Galgani, 2016). In our case, the abundance of microplastics in the Ghar el Melh lagoon is likely attributed to secondary sources, such as the degradation of larger plastic items (e.g., fishing nets, plastic packaging). The sheltered nature of the lagoon, along with local activities like agriculture and fishing, may further contribute to this pollution. In contrast, for Kalaât el Andalous, the proximity to urban and industrial centers suggests that pollution may arise from both primary sources (e.g., plastic pellets, small objects) and secondary sources, such as nearby rivers or rainwater runoff, which transport microplastics into the sea. (Khsiba et al., 2021).

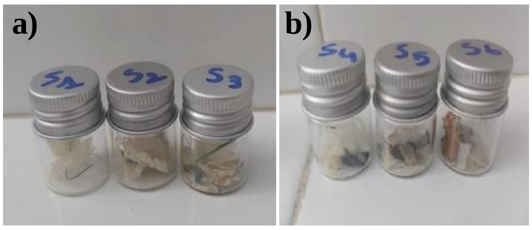

Figure 10. (a) Microplastics collected in Ghar el Melh, (b) Microplastics collected in Kalaât el Andalous.

5.2. Comparative analysis of the characteristics and abundance of microplastics in Tunisian and global aquatic ecosystems

Our study found that the abundance of microplastics in the Gulf of Tunis ranged from 66.395.66 to 104.336.04 items/km², highlighting the variability of microplastic pollution across different marine ecosystems (**Figure 9.a**). In our specific case, the first transect near to the Ghar el Melh Lagoon demonstrates an average of 241192.41196 items/km^2, which is more loaded with microplastic than that of Kalaât el Andalous with 108401.08401 items/km^2 (**Table 2**). The Lagoon of Kalaât el Andalous receives pollutants from water discharges, plant treatment, irrigation water, and the oued of Medjerda, impacting its ecological state (Khsiba et al., 2021). Besides, the size distribution of microplastics in water samples suggests a connection between the quantity of these particles and their size (**Figure 9.b**).

Similarly, global studies, such as those in the Mediterranean region or other coastal zones, allow us to contextualize our findings more comprehensively. These additions enhance the understanding of the unique characteristics of microplastic pollution in the Gulf of Tunis while situating our work within a broader scientific framework. For instance, studies conducted in the Southern Mediterranean, Monastir Bay reported abundance values ranging from 62.095.03 to 260.979.12 items/km² (Jaziri et al., 2022), which are somewhat comparable, suggesting a similar level of microplastic contamination in coastal zones. In addition, in the Gulf of Gabes (Southern Mediterranean), the abundance values were found to range from 77.110 to 312.887 items/km² (Ben Ismail et al., 2022) showing a higher presence of microplastics, particularly in more heavily impacted areas. Moreover, in the broader South-Western Mediterranean Sea, microplastic concentrations ranged around 1.01 x 10^5 ± 3.8 x 10^4 items/km², (Setiti et al., 2021) indicating a slightly higher abundance when compared to the Gulf of Tunis, depending on the sampling areas. In the Central and Western Mediterranean Sea, a broader range of 8.999 to 1.164.403 items/km² was observed, reflecting the variability of pollution levels, influenced by local environmental and anthropogenic factors (Digka et al., 2018). Additionally, in the Northern Ionian Sea (Greece), microplastic abundance reached extreme values of 0 to 1.610.000 items/km², (Galli et al., 2022) showing the highest recorded densities in the Mediterranean region. This suggests a significant degree of variability, potentially due to differing methodologies, types of microplastics sampled, and local environmental factors, as mentioned in (**Table 3**). These comparisons with studies conducted in various Mediterranean regions support the idea that while microplastic pollution is a widespread problem, its intensity and distribution can vary considerably depending on local environmental conditions and human activities.

5.3. Parameters affecting the abundance of microplastics in aquatic matrices

According to (Wakkaf et al., 2020), a study was carried out to assess the effects of environmental factors on the distribution and abundance of microplastics in the surface waters of the Bizerte lagoon, revealing a significant amount of microplastics. This confirms that this area has been subjected to pollution and holds significant importance due to its dense coastal population, leading to substantial pressures on the marine ecosystem. The high concentration of microplastics can be clarified by the hydrodynamic simulation, revealing a noticeable rise in the exchange of water between the sea and the lagoon. This correlation appears to be shaped by various environmental deterioration processes such as thermal, chemical, microbial, and mechanical photooxidative forces (Ramaremisa et al., 2022). The comparison between the two sites highlights the impact of environmental and anthropogenic factors on microplastic distribution. This analysis underscores the necessity of adopting site-specific strategies for managing microplastic pollution, taking into account variables such as water circulation patterns, local human activities, and the nature of pollution sources. Both sites are influenced by a combination of primary and secondary sources. However, Ghar el Melh may be more significantly impacted by the degradation of larger plastic objects. Its semi-enclosed nature, limited water exchange, and accumulation of plastics from local sources (Rasmussen et al., 2009) likely contribute to higher microplastic concentrations and long-term accumulation. Additionally, biofouling may be more pronounced in this area, accelerating the fragmentation of larger plastic debris into smaller particles. Among these, high-density polyethylene (HDPE) commonly used in plastic bottles, food packaging, and containers emerges as the dominant polymer identified in the samples. Other polymers, such as polystyrene, polypropylene, polyethylene, nylon, and polyester, are also found in substantial quantities, reflecting further pollution indicating that household plastic waste, industrial activities, and fisheries-related waste are the main sources of pollution (Osman et al., 2023). While, Kalaât el Andalous, which is affected by direct urban and industrial pollution has a lower abundance of microplastics than Ghar el Melh, due to continuous water exchange and higher dilution of contaminants (Khsiba et al., 2021). Films and fragments are also present, but to a lesser extent compared to Ghar el Melh, given the more dynamic water conditions. HDPE, a specific form of polyethylene, remains one of the most abundant polymers in this environment, although its profile may vary slightly depending on pollution sources. In this study, HDPE accounted for the majority of microplastic particles, with proportions of 67% for T_1 and 33% for T_2. Similarly, (Jaziri et al., 2022) mentioned that the highest percentage of HDPE polymers was noted for 43.47% in T_1 and 63.77% in T_2. Furthermore, (Digka et al., 2018) found that polyethylene, encompassing its different forms, constituted 67.2% of the identified microparticles. Consistent results were reported by (Ben Ismail et al., 2022), where PE, including HDPE, dominated the composition of microplastics, reaching 75% in the first campaign and 67% in the second. Furthermore, (Galli et al., 2022) identified PE as the most abundant polymers with 78% of the total. These results highlight the persistent prevalence of polyethylene, especially in its high-density form, in aquatic ecosystems. This dominance is influenced not only by the intrinsic properties of the polymer, such as buoyancy and durability, but also by hydrodynamic and environmental parameters that affect its distribution. Wind speed and direction, for instance, can impact the dispersion of microplastics on the surface, leading to their accumulation in specific areas (Kye et al., 2023). Similarly, boat speed could influence the efficiency of microplastic collection using the manta net. To account for such operational variables and minimize sampling bias, monitoring of distances travelled and collection times is essential to ensure the representativeness and consistency of samples. Moreover, to accurately assess microplastic pollution, it is important to consider the scale and frequency of sampling. Sample size can also reflect the spatial and temporal variability of microplastic pollution. Understanding this variability requires standardized sampling protocols and repeated measurements across different locations and time periods. These factors could influence the abundance and distribution of microplastics; therefore, more frequent sampling during different seasons would provide a better understanding of seasonal fluctuations in microplastic concentrations (Chouchene et al., 2023).

6. Conclusion

Our study aimed to assess the abundance, composition, and sources of microplastics in surface waters, with a particular emphasis on understanding their environmental impacts. The findings revealed a high abundance of microplastics, as confirmed by microscopic analysis, which highlighted clear signs of fragmentation caused by environmental conditions, biofouling, and adsorption of other compounds on their surfaces. Films emerged as the most dominant form in surface water samples, suggesting secondary sources as the main contributors to microplastic pollution. Among the identified polymers, HDPE was the most prevalent, pointing to specific plastic debris as a potential source in the study area. The high abundance of polymers in surface waters underscores the persistence of contaminants that are not fully biodegradable and are resistant to removal by typical water treatment processes. These findings highlight the potential risks posed by microplastics over the medium to long term, including bioaccumulation and the formation of new mixtures in aquatic environments. To address this, future studies should focus on identifying the precise sources of microplastics and developing innovative and eco-friendly alternatives to mitigate their prevalence. Additionally, enhancing public awareness and improving waste management practices could contribute to reducing microplastic pollution in aquatic ecosystems.

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Table 3 - Recent data on the abundance of microplastic in water: (from 2014 to 2024), sampling method and analysis studies (Aragaw., 2021).

Country	Sampling method / Analysis studies	Abundance	References
In the open ocean (The Spanish circumnavigation expedition Malaspina 2010)	Microplastic sampled at 141 sites across the oceans. Floating plastic was collected with a neuston net (1.0-×0.5-m mouth, 200-µm mesh) towed at 2-3 knots, 10-15 min (total tows 225). Filtration of 0.2-mm. A dissecting microscope. Raman spectroscopy	North Pacific Ocean :12.4 kilotons North Atlantic Ocean:6.7 kilotons Indian Ocean : 5.1 kilotons South Atlantic Ocean: 5.4 kilotons South Pacific Ocean :5.6 kilotons	(Cózar, A et al.,2014)
Norway, south and southwest of Svalbard	A Manta net, Stainless steel sieve (250 µm). Vacuum filtration on GF/C filter paper (diameter 47 mm). Stereomicroscope.	Microplastic abundance ranged between 0 and 11.5 particles per m^3, and averaged 2.68 (± 2.95 SD) particles per m^3	(Lusher et al., 2015)
South Africa, KwaZulu-Natal	Net (300 µm mesh), filtered through 1000,500 and 250 µm Sieves FTIR-ATR.	159.9±271.2 particles per 500 ml	(Naidoo et al., 2015)
South Africa, Southeastern Africa	Using a type net (80 µm) for sampling Visually under a microscope, ATR-FTIR.	257.9±53.36 to 1215±276.7 particles m^{-3} for water	(Nel et Froneman, 2015)
South Africa, Cape Town	Continuous pumping through stainless steel pipes - Filtration - glass microfiber Microscopic visualization.	1.15±1.45 particles m^{-3}	(Daana et al., 2016)
Canada	Manta trawl with a mesh size of 333 µm, 295 cm long, an aperture width of 61 cm, a height of 18 cm, filtering through a 355 µm sieve using MilliQ water, 70% ethanol, viewing with a dissecting microscope, scanning electron microscopy and energy dispersive Xray spectroscopy	The Red River : 632,489 microplastics/km^2, The Assiniboine River : 812,672 microplastics/km^2	(Warrack et al., 2017)
Vietnam	Using a 300 mm mesh size net exposed in surface water, image analysis software and FTIR microscopy	For fibers: 172,000 to 519,000 items m^{-3} For fragments: 10 to 223 items m^{-3}	(Lahens et al., 2018)
Nigeria, Nwangelo	Grab sampling technique at the depth of 0-3 cm with a "W" shaped design – Filtration Visual microscope.	3,487 items/m2; 469±153.33 items/ m2 (downstream); 85.8±174.94 items/ m^2 (midstream); 211.4±109.84 items/m2 (upstream)	(Ebere et al., 2019)
South Africa, Northwest	Neuston net (mesh size of 300 µm), and bulk water sampling Visual microscope.	bulk water:0.23±0.27 items L^{-1}; net: 0.04±0.16 items m^{-2}	(Weideman et al., 2019)
South Africa , Johannesburg	Direct fetching with a container for a water sample Microscopic visualization.	Mean of 705 particles m^{-3} in water	(Dahms et al., 2020)
Nigeria, Yenagoa	Tefon pump through stainless steel mesh, with a grab on the top 5 cm Microscopic visualization.	1004 to 8329 items m^{-3} for the dry season and 201to 8369 items m^{-3} for the raining season, respectively, for water;	(Babalola et al., 2020)
South Africa, Cape Town	Neutron net (a 300 mm) trawled by a boat Pyrolysis-GC-TOF- MS SEM-EDS, FTIR, TGA.	-	(Vilakati et al., 2020)
South Africa	Bulk and neutron nets sampling Microscope visualization, Raman spectroscopy.	2.3 ± 7.2 microfbers L−1 in the wet season and 1.4 ± 2.6 microfbers L−1 in the dry season, and 0.2 ± 0.2 fragments L^{-1}	(Weideman et al., 2020)
Tunisia, (Bizerte lagoon, Southern Mediterranean Sea)	Sea water samples taken at each station were pumped, separately, from the top 25 cm surface water layer, using a water pump on board a small boat, and filtered through a cylindrical stainless-steel filter with a mesh size of 300 µm and a diameter of 4.5 cm FTIR -ATR.	Average concentration of total MPs was found to be 453.0 ± 335.2 items m^{-3}	(Wakkaf et al., 2020)

Country	Sampling method / Analysis studies	Abundance	References
Tunisia, Gulf of Gabes	Using a trawl net (0.6 m in width x 0.2 m in height) mounted with a 200-µm mesh size. A flowmeter equipment (HYDRO BIOS) was fixed in the middle of the trawl frame (CTD) profiler (SeaBird Electronics Inc., SBE19plusV2 SN 6573). Density separation with NaCl solution (1.2 g L −1) 30% H2O2 iron (II) sulfate (0.05 M) filtration with a glass fiber filter (GF/F Whatman, 47-mm diameter, 0.7-µm pore size) using a vacuum filtration system Visually under a microscope, FTIR, Modelling systel ROMS	The total number of MPs collected was 1382 MP particles. The MP abundance varied between 25,471 items/km2 (s5) and 111,821 items/km2 (s2) with a mean value of 63,739 items/km^2. (S4), located 24 km off the coast, showed a high MP abundance with 93,202 items/km^2.	(Zayen et al., 2020)
upper ocean and the Laurentian Great Lakes	Using surface net tows or continuous seawater intake. Identification by the naked eye and/or using a stereomicroscope, µFTIR, Raman spectroscopy	24.4 trillion pieces (8.2 × 104 ~ 57.8 × 104 tons)	(Isobe et al., 2021)
Tunisia, Monastir	Using a 200 µm Manta net (0.6 m width 0.2 m height) - use filter blanks in parallel to verify airborne contamination Visually under a microscope, FTIR,	T1 : 62095.03 items/km^2 T2 :260979.12 items/km^2	(Jaziri et al., 2022)
The Mediterranean Sea, Saronikos Gulf GS LIgurian Sea LS , Gulf of Lion GL Gabes Gulf GG	Manta net with different mesh and open mouth size: a 100 and 330 µm manta net (W0.70xH0.40m) in LS and GL; a 330 µm manta net (W0.60xH0.24m) in GS and a trawled net (W0.60xH0.20m) mounted with a 200 µm mesh size in GG. Filtration Microscope, FTIR, Modelling tools.	GG(0.073-0.310 items/m^2), LS(0.061-0.134 items/m^2), GS(0.047- 0.080 items/m^2), GL(0.029-0.032 items/m^2)	(Tsiaras et al., 2022)
Tunisia, Gulf of Gabes southern Mediterranean Sea	Using a 200 µm mesh manta net. The Samples were then transferred to 500 mL glass containers using a 200 µm mesh stainless steel screen, quenched with 70% ethanol, and transported to the laboratory Microscope, FTIR-ATR, modelling tools	The average abundance is between 312,887 and 77,110 items/km^2 .	(Ben Ismail et al., 2022)
South Africa, Vaal River in Johannesburg	Using a 55-µm plankton net (Hydro-bios). Raman analysis. A scanning electron microscope (SEM) stereomicroscope.	Average abundances of 0.61 ± 0.57 particles/m^3	(Ramaremisa et al., 2022)
the Mediterranean Sea Gulf of Lyon, Tyrrhenian Sea, and Eastern Basin	Sampling was conducted using a 4.4 m long manta net (mesh size: 333 µm; net opening: 16 × 60 cm), in 154 sites which were selected based on ocean colour satellite images supplied by ACRI-ST and analyzed with the Mercator circulation model. At each site, the manta net was towed on the sea surface for ca. 60 min behind the boat at an average speed of 2.5 knots, enabling thus the filtration of about 507 m3 of seawater. Stereomicroscope /Fourier Transform Infra-Red (FTIR)	58,043 ± 36,010 particles/km^2.	(Kedzierski et al., 2022).
South Atlantic Gyre	Using in situ high volume filtration, Manta net and MultiNet sampling, combined with stereomicroscope and micro Fourier transform infrared imaging	244.3 pieces per cubic meter [n m−3]) of small microplastics (SMPs) ~600 g per km^2	(Zhao et al., 2022)
Eurasian Arctic, Kara Sea Western Kara Sea plume waters W. N. Zemlya Current Northern Kara Sea	Neuston net (40 x 60 cm opening, nylon material, 0.333 mm mesh size) trawling over the sea surface at low vessel speed (2-3 knots) for about 20 min. 20 cm of the net opening was submerged	Kara Sea 0.93 ± 0.73 items/m3 Western Kara Sea (0.023 ± 0.011 items/m3) plume waters (0.008± 0.009 items/ m3) W. N. Zemlya Current (0.005± 0.009 items/m3). Northern Kara Sea (0.019 ± 0.029 items/m^3)	(Berezina et al., 2023)
South Africa, Western Cape,	Water samples of 30 L were collected and filtered in triplicate of 10 L through a 250 µm tube. trellis on site using a metal bucket. An additional 12 L sample was taken and filtered in triplicate of 4 L through 20 µm mesh in the laboratory. FTIR ATR.	Spring samples had the highest PM occurrence (5.13 ± 6.62 MP/L) and the lowest, in autumn (1.52 ± 2.54 MP/L).	(Apetogbor et al., 2023)
South Africa, Western Cape coastline	Water samples were filtered on site with a 250 µm metal filter. sift then store. The samples were then transferred to a pre-cleaned glass jar and digested in 10% (w/v, Merck, South Africa) potassium hydroxide (KOH) (1:2 ratio) for 24 h at 50 ◦C. Filtered using a Buchner funnel system and vacuum pump. rinsed 3 times with osmosis water filtered at 10 µm and filtered on the same 20 µm mesh. Stereomicroscope / FTIR-ATR	A total of 1840 MP particles were recorded in water samples (1.52 ± 0.20 particles/L).	(Julius et al., 2023)

Country	Sampling method / Analysis studies	Abundance	References
Europe along the land-sea continuum (along nine major rivers in Europe–Thames, Elbe, Rhine, Seine, Loire, Garonne, Ebro, Rhone, and Tiber)	Large microplastics (330 µm to 5 mm): using a 330-µm mesh size manta trawl (aperture of 30 × 80 cm, 2.5 m long nylon net, and 30 × 10 cm2 weighted cod end). 2.0 knots & 60 min in seawater and 10 min in rivers. The volume of sampled water was measured using a mechanical flow meter located at the center of the net aperture (Hydo- bios, Kiel, Germany). Particles with approximately 1 to 5 mm in size accumulated in the final volume of 1.0 l of the collector were transferred in glass Petri dishes, observed under a binocular magnifying glass, and sorted using alcohol/flame sterilized forceps. An attenuated total reflection-Fourier transform infrared spectrometer (ATR-FTIR Vertex70v, Bruker, ATR Golden Gate) Small microplastics (SMPs; 25 to 330 µm): The 25-µm nylon net (aperture of 32 × 82 cm with two floats placed on both sides for buoyancy, 2.5 m long net and 30 × 10 cm2 weighted cod end) was deployed for 10 min in seawater and 2 min in rivers at an approximately 2.0 knots speed. A mechanical flow meter located at the center of the aperture (Hydo-bios, Kiel, Germany) was used to determine the volume of sampled water. The net was thoroughly rinsed with on-site water and the collector was rapidly transferred to a calcinated 1-L glass jar equipped with a metallic cap in order to prevent any airborne contamination. A new method was developed to analyze the small-microplastic fraction (25-330 µm) by pyrolysis coupled to gas chromatography and tandem mass spectrometry (Py- GC-MS/MS).	-	(Ghiglione et al., 2023).
The Brantas River estuary	Plankton net, Filtration through an 80 m mesh. sample preserved with 70% ethanol solution and tightly closed, destruction of organic matter using 30% H2O2. filtration using Whatman grade 42 filter paper with a pore size of 2.5 µm. Filtration by vacuum filtration (buchner funnel filtering)	In the dry season: sta 1 0.8 particles/L, sta 2 1.25 particles/L, and sta3 1.02 particles/L. In the rainy season, : sta 1 0.9 particles/L, sta 2 1.24 particles/L, and sta3 1.17 particles/L.	(Ekawati et al., 2024)
South Africa, Vaal River	Using a plankton net with 0.25 m, 50 cm, and 0.055 mm diameter, length, and mesh size, respectively. Digestion by peroxide oxidation process (WPO) using Fenton's reagent (0.07 M $FeSO_4 \cdot 7H_2O$ catalyst and 30% (v/v) H_2O_2). NaCl (1.2 g/mL), NaI (1.8 g/mL), GF/A flter papers (Whatman® : 47 mm diameter, pore size: 1.6 µm), stereomicroscope (Nikon MET SMZ745T, Japan) at 50x magnification, NIS Elements-D imaging software Version 5.30, Raman spectroscopy, filter Milli-Q® Type 1 Ultrapure water, rinsed with filtered ethanol.	0.68±0.64 particles/m^3	(Saad et al., 2024)
Akmola Region (Kazakhstan)	manta trawl nets, meshes ranging from 300 to 390 µm , Photometric measurement, Microscope (DTX 500 LCD Levenhuk with photo and video registration), Analytical electronic scales: AX-200 Shimadzu (measurement accuracy 0.0001 g); Stainless steel sieves with mesh sizes: 3, 2, 1, 0.3, 0.175 mm; Electric dry-air thermostat: TS-1/80 SPU (maximum deviation of the average temperature not more than ±1◦C, maximum deviation of the temperature at any point ±0.4 ◦C); Water bath: "Ekros" model 4310; Ultrasonic bath: UZV-4.0 "Sapphire" with a digital thermostat (temperature range from 15 to 70 ◦C, ultrasound frequency 35 kHz, timer from 1 to 99 min); Filters for quantitative analysis: Whatman No.2; centrifuge: Opn-3.01 "Dastan" centrifuge with rotation speeds of 1000, 1500, and 3000 rpm; Set of areometers; 5.75 M ZnCl solution, FTIR.	In spring 1.2 × 10−1 particles/dm^3 In autumn 4.5 × 10−1 particles/dm^3	(Salikova et al., 2024)
China	Water samples were collected using a 5 L stainless steel tank at each site and filtered through a 20 mm stainless steel sieves, iron shovel, 30% H2O2, a 0.45 µm (GF/F, 47 mmØ, Whatman) filter paper, stereo microscope (SMZ25, Nikon,), Raman spectroscopy.	1033 to 8333 items/ m^3	(Zhang et al., 2024)

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