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Research Paper

Assessment of microplastic pollution in sediments and water of River Soan, Punjab, Pakistan

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ABSTRACT

Plastic debris represents a significant environmental threat, with microplastics (MPs) posing a major risk to water quality. This research focuses on assessing MPs' presence in water and sediment samples from the River Soan during two distinct seasons: winter and summer. Analysis of the samples taken from seven locations showed that there were 318 microplastic particles per 0.25 m^2 in winter and 500 particles per 0.25 m^2 in summer. In the sediment samples, concentrations of 2,466 and 2,341 microplastic particles per 20 g of dry sediment were observed during the winter and summer seasons, respectively. A t-test revealed a statistically significant difference between the concentrations of MPs in both water and sediment samples. Furthermore, ATR-FTIR analysis confirmed the presence of various polymers, including PET, PVC, PS, nylon, Acrylonitrile Butadiene Styrene, Poly Methyl Methacrylate, Polycarbonate, and Urethane compounds. The findings can aid in enhancing waste management practices in the River Soan area and in modeling the transport dynamics of microplastics in other river systems by incorporating water quality parameters and basin characteristics.

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1. INTRODUCTION

Microplastics (MPs) are plastic particles that are less than 5 mm in size and are susceptible to breaking down into even finer fragments [1]. Microplastics are now recognized as being extensively distributed across the environment, with evidence of their presence even in remote locations like Mount Everest. These tiny particles can be classified into primary and secondary microplastics and differentiated based on attributes such as color, shape (like spherical or flat), and composition (fragment, film, or fiber) [2]. As per recent studies, MPs can be found in sediments, rivers, wastewater, the air, and soil in addition to the ocean [3] as a result of improper handling of plastic waste, technological shortcomings in the methods used to treat wastewater in cities and businesses, and slow material deterioration [4].

River sediments function as reservoirs for microplastic pollutants, serving as both a sink where these particles accumulate and a potential source for further dispersion into the environment [5]. Plastic waste has been dumped into the environment since the 1960s. Since then, rivers all over the world have carried this novel, synthetic element, leading to several issues with aesthetics, ethics, the economy, and the environment [5]. Each year, rivers around the world transport an estimated 1.2 to 2.4 million tons of floating plastic pollutants from inland regions to the oceans. Sources of microplastic pollution include atmospheric deposition, beach litter, waste from ship maintenance and harbor activities, along with personal care products, industrial cleaning processes, physical wear of plastics, synthetic textiles, and tire abrasion (Figure 1) [6].

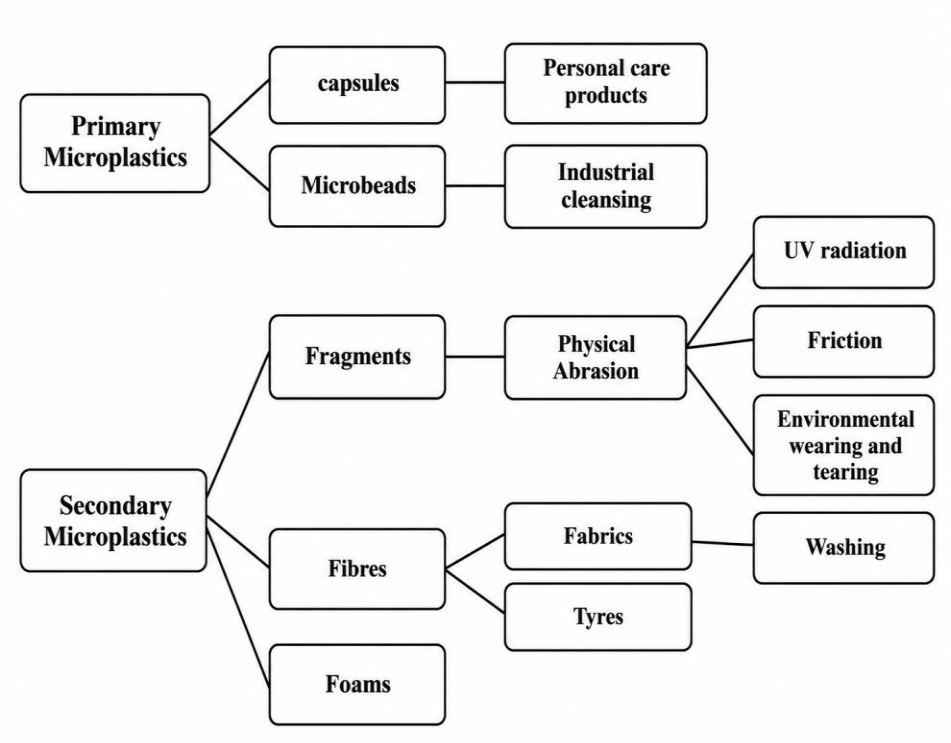


Figure 1. Summarizes the Types of Microplastics and their Sources in the Environment [7]

MPs are transferred between trophic levels, impacting metabolic pathways in various organisms [8]. Because of their abundant presence and tiny size (Figure 2), MPs can easily enter the human body through the food chain [9]. The bio-persistent characteristics of MPs can result in toxicity, leading to inflammation, oxidative stress, apoptosis, and necrosis, which may contribute to carcinogenesis and tissue fibrosis following exposure through oral ingestion or inhalation. [10]. Various marine organisms, including fish, zooplankton, phytoplankton, and seabirds can ingest MPs.. MPs clog digestive pathways if ingested leading to the death of organisms [11].



Figure 2. Summarizes the Relative Sizes of Plastics Available for Commercial and Industrial Uses

2. RELATED WORK

MPs have been confirmed in table salt in China [12], mineral water [13] and honey [14]. This raises concerns about the prevalence of microplastics in our environment. Global studies on MPs (Table 1) have demonstrated their presence in both the surface water and sediments of rivers. Research conducted on the Crocodile River in South Africa revealed seasonal variations in microplastic concentrations [15]. Similarly, Rawal Lake in Pakistan has shown clear evidence of microplastic contamination in both its water and sediment [16]. These findings highlight the necessity for thorough assessments across various river systems. Therefore, the present study aims to evaluate the abundance and distribution of microplastics in the sediments and water of the River Soan and to propose effective mitigation strategies to combat microplastic pollution.

Table 1. Microplastic Pollution in Water Bodies Around the Globe

Region	Quantity Detected	
San Francisco Bay, California, USA	700,000 particles/km2	(Sutton et al., 2016)
Lake Winnipeg, Canada	4.74^9 km2	(Anderson et al., 2017)
Lake Superior, Canada	4.43^8 km2	
Lake Huron	1.66^8 km2	
Lake Erie	2.72^9 km2	
Qinghai Lake, China	2-15 items/ km2	(Xiong et al., 2018)
Northern Pacific Ocean	8-9200 items/m3	(Desforges et al., 2014)
Dutch River	100-3600 particles/kg	(Leslie et al., 2017)
River Danube	200g/L	(Lechner& Ramler, 2015)
Lake Hovsgol, Mongolia	20,264 particles/km2	(Free et al., 2014)
Table Salt, China	550-681 particles/kg (sea salt) 43-364 particles/kg (lake Salt) 7-204 particles/kg (rock salt).	(Yang, Shi, Li, Li, Jabeen, & Kolandhasamy, 2015)
Urban Surface Water, Wuhan	1660-8956 items/m3	(Wang et al., 2017)
Changiiang Estuary, China	20-340 items/kg	(Peng et al., 2017)
Verbanad Lake, Kerela, India.	96-496-2.	(Sruthy & Ramasamy, 2017)
Antua River, Portugal.	5-8.3mg m-3- 58-51mg m-3.	(Rodrigues et al., 2018)

2.1 Study Area

The River Soan is located in Northern Pakistan, extending between longitudes 71°45' and 73°35' east and latitudes 32°45' and 33°55' north within the Potohar region. This river is fed by several tributaries of the Indus River, including the Korang River, Dharab Nala, River Ling, and Lai Nullah. It serves as a tributary of the Indus River and comprises five major streams, with a total length of 274 kilometers. Approximately 900 hectares of its riverbank are suitable for irrigation. The river also supplies water to

Khanpur Dam, Simly Dam, and Rawal Dam. In the vicinity of the river, over 1,200 industrial units operate, including pharmaceutical companies, oil and ghee manufacturing plants, polishing facilities, as well as marble and flour mills [17].

3. METHODOLOGY

The study emphasizes the visualization, identification, and characterization of microplastics collected from various sampling sites. Comparisons of concentrations were performed using t-tests and ANOVA. A schematic representation of the methodology employed is shown in Figure 3.

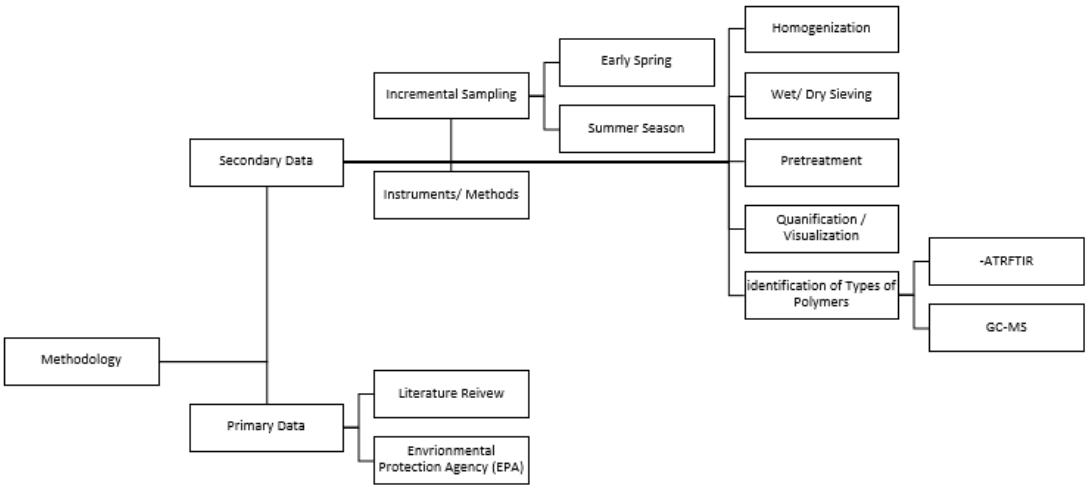


Figure 3. Methodological Framework for Sampling, Pre-Treatment, Quantification and Polymer Identification of Microplastics

During late winter and summer of 2019, seven sampling sites (Figure 4) were monitored to determine the concentration of MPs in both running water and sediment. A bulk sampling method was employed, following the approach outlined by Hidalgo-Ruz et al. [18] to collect samples from the selected sites. The methodology developed by Di & Wang. (2018), Xiong et al. [19], Zhang et al. [20] was adapted with minor modifications. Wet sieving, digestion, and density separation of the samples were conducted according to the protocol established by Masura et al. [21]. The characterization of MPs is significantly influenced by the pore size of the filters and sieves utilized [22]. Consequently, the results from optical microscopy were categorized based on size ranges (>100µm, >1mm, and larger).

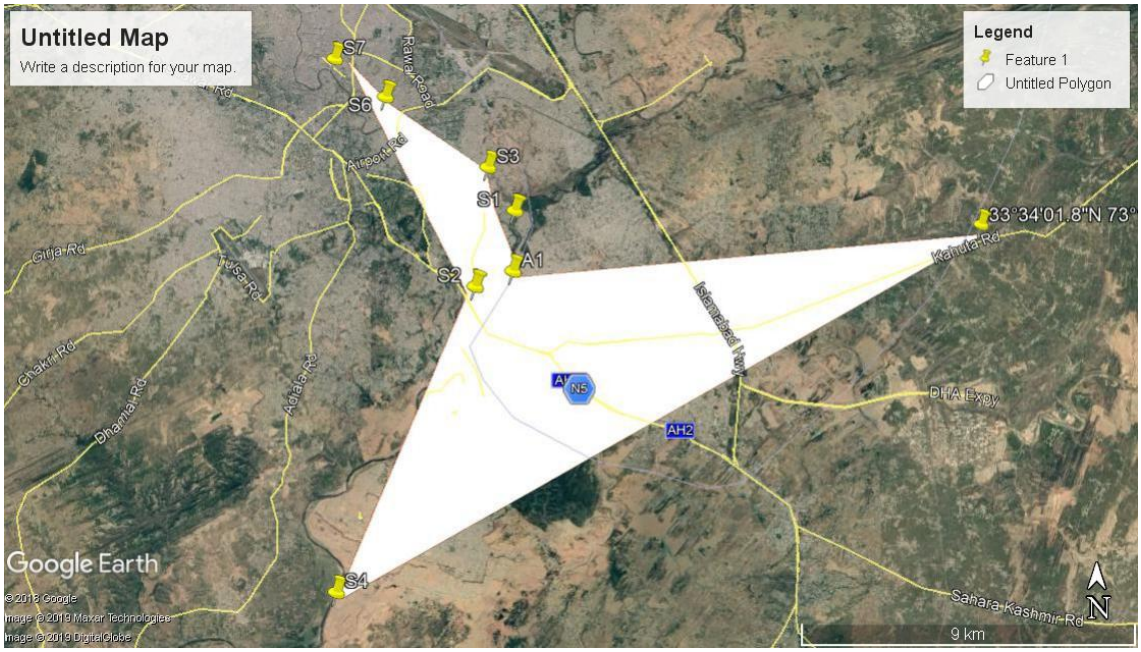


Figure 4. Map of the Sampling Sites of River Soan Selected for the Study

3.1 Sediment Samples

The collected sediment samples were dried at 60°C to eliminate moisture content, as suggested by Qiu et al. [23] to prevent the growth of microorganisms. The samples were then passed through 5 mm and 2 mm stainless steel sieves to minimize the presence of macroparticles. Any particles suspected to be larger than 5 mm were isolated and subjected to the Hot Needle Test, following the method described by Qiu et al. [23] before being examined under a microscope. A 20 g sample was taken and dissolved in distilled water at a 1:4 ratio. To remove organic material, 30% H_2O_2 was added to the sample. The extraction of microplastics (MPs) from sediment samples was conducted using a density separation process [21]. The digested sample was combined with a saturated salt solution (1.2 g/ml NaCl) and shaken for a period. Sodium chloride was chosen for this process due to its low environmental toxicity [22]. This procedure was repeated three times to ensure maximum recovery of MPs from the samples.

3.2 Water Samples

A 250 ml liquid sample was filtered through a 5 mm sieve to eliminate any macroparticles. Then, 20 ml of 30% H_2O_2 was added to the sample, which was stored in the dark for five days at room temperature to digest organic contaminants. Floating particles were collected, while the settled portion was passed through a sieve and manually examined for any remaining particles.

3.3 Visualization of Microplastics Using Optical Microscopy

The subsequent step in the determination of microplastics (MPs) involved identifying the shapes and types of MPs present in the samples. For this purpose, visual sorting was conducted as described by [18]. The digested samples were filtered using Glass Microfiber Filters (GF/F 47 mm). The microscope was set to a magnification of 10X. After filtering, each filter was placed in a clean petri dish and allowed to air dry. All filters were examined twice using a Novel compound microscope (OPTIC 1000x) to ensure the accuracy of the readings. The abundance of microplastics was calculated based on the number of particles per square meter.

3.4 Criteria for Microplastic`s Identification

Microplastics observed under the microscope were classified into several categories: films, fibers, fragments, microbeads, and foams. The identification criteria included their size (less than 5 mm), shape, and overall appearance [18].

3.5 Microplastics Identification and Quantification

The determination of MPs in water was conducted using two techniques. Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) was employed to identify the types of bonds present in the polymer components based on their absorption spectra (cm^{-1}). The specific type of polymer in each sample was verified by comparing the IR spectrum with the FTIR polymer spectrum library referenced in the literature (Löder et al., 2015) (Löder et al., 2015). Additionally, Gas Chromatography-Mass Spectrometry (GC-MS) was performed on selected particles after the density separation process to acquire information regarding the type, mass, and structure of the involved polymers [24].

3.6 Data Analysis

A t-test and ANOVA were conducted on the data from the two seasons using XLSTAT for the purpose of tabulation and presentation of the results.

4. RESULTS AND DISCUSSIONS

The study focused on identifying the shape, size, and type of microplastics detected during analytical testing. Microplastics were classified into categories such as foams, fibers, films, fragments, and microbeads, which were observed and documented after a detailed examination of the filters using a compound microscope. However, large clay particles accumulated on the filters, which hindered the accurate identification of the shapes of the particles, as illustrated in Figure 5. To mitigate this issue, some of the clay was carefully scraped off the filters and analyzed separately.

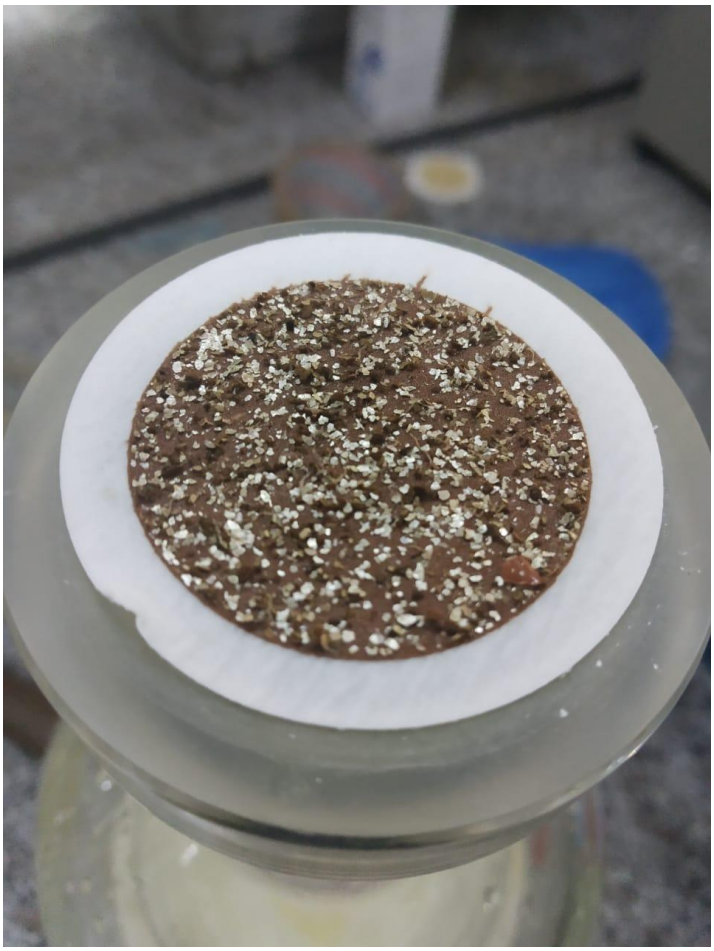


Figure 5. Sample Collection from the Selected Sites

Figure 7 highlights key morphological features of microplastics obtained from water samples in the study. Samples collected during winter (January-February) were compared with those collected in summer (May-June). The relative abundance of the microplastic types was as follows: spheres > fragments > foams > films > fibers. The results suggest that microplastics were more prevalent in the sediment than in the water at this site during both seasons. This could be attributed to factors such as the size of clay particles, pore size, particle distribution, physical and environmental impacts, proximity to localities, and the form in which the microplastics are deposited in the medium (Biswas et al, 2018). In contrast, water is continuously flowing, causing particles with greater masses to settle into the lower layers (sediments). This explains why a higher number of spheres and fragments were found in the sediments compared to the water. The highest concentration of microplastics was recorded at Site 6 (Lai Nallah), while Site 5 (Soan River Bridge) experienced a significant increase in MP concentration during Season 2. The longest fiber, measuring 20 microns, was observed at Sampling Site 2 (Soan Bridge, GT Road) in the summer. Additionally, the largest foam among all samples, measuring 2 microns, was found at Site 3 (Lai Nallah tributary) during the summer (Figure 6).

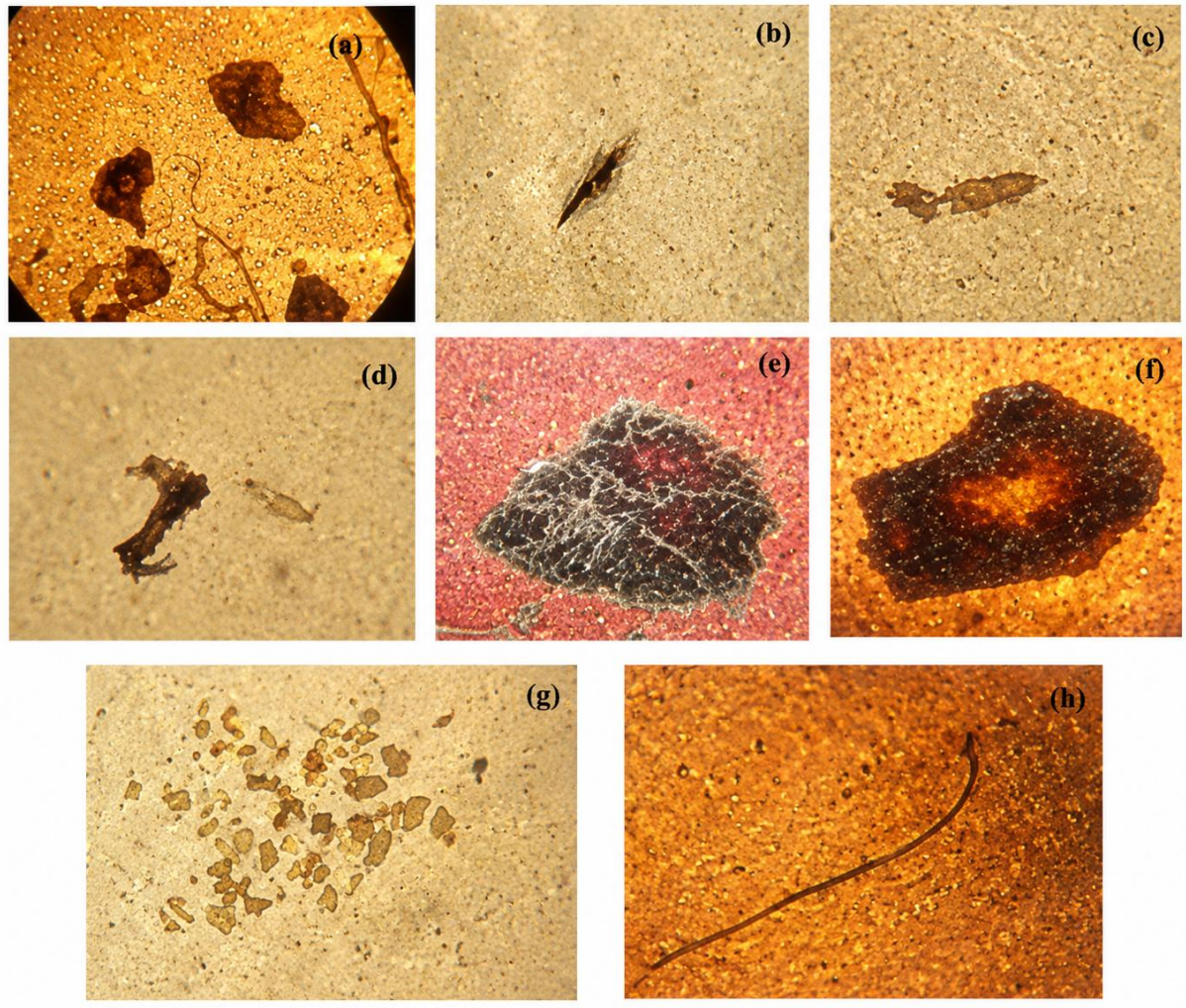


Figure 6. (a) Microfibre; (b), (c), (d), (e) Microfoams; (f), (g) Fragment; (h) Film

4.1 Quantification of Microplastics in Water Samples

Figure 7 illustrates the graphical representation of microplastics (MPs) found per 250 ml of water samples. The highest concentration of MPs was recorded at Site 5 during Season 1 and at Site 6 in both Seasons 1 and 2. The most prevalent type of MPs was spherical, with 567 particles per 250 ml water sample at Site 5 and 433 particles per 250 ml water sample at Site 6.

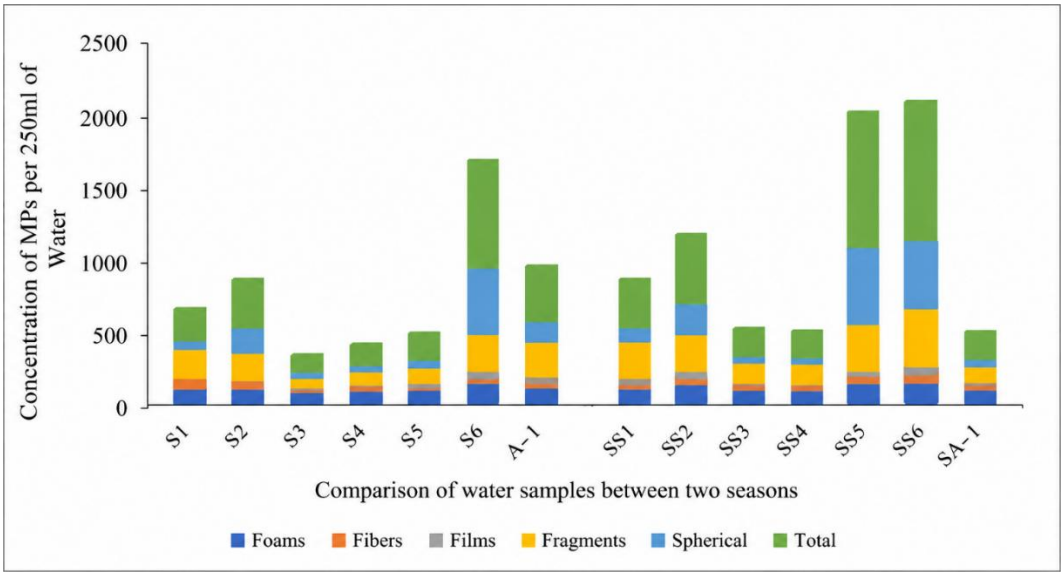


Figure 7. Salient Features of MPs Found per 250ml of Water

4.2 Quantification of Microplastics in Sediment Samples

Figure 8 displays the total number of microplastics (MPs) found per 20 g of sediment samples. The highest concentration of MPs was detected at Site 6 during both seasons. A general decrease in the concentration of microparticles was observed across all sampling sites in Season 2. The most abundant form of MPs was spherical, with quantities ranging from 561 to 4,708 particles per 20 g of sediment.

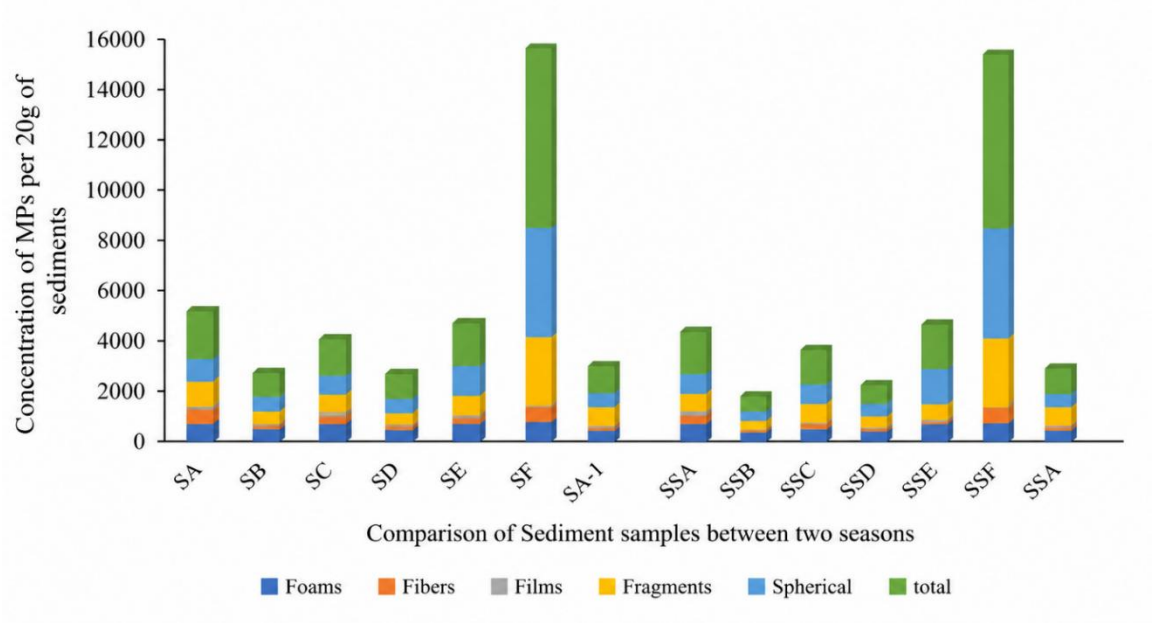


Figure 8. Salient Features of MPs Identified per 20g of Sediments

The average concentration of microplastics (MPs) in sediments was 2,466.2 particles and 2,341.7 particles per 20 g of sediment (Figure 8). The observed decline in MP concentration in the sediments during Season 2 may be attributed to consistent rainfall or alterations in commercial activities throughout the seasons.

4.3 Comparison of Results between Water and Sediments

Figure 9 illustrates the differences in average concentrations of MPs between water and sediments across the two seasons. The average concentration of MPs in water was 318.6 particles per 0.25 m^2 in

Season 1 and increased to 500 particles per 0.25 m^2 in Season 2. In contrast, sediments from Season 2 contained a higher number of particles per unit area compared to those from Season 1. The average number of particles in winter was recorded at 2,466 per 20 g, while there was a slight reduction in summer, with an average of 2,341 particles per 20 g. The highest concentration of microplastics was observed at Site 6 in Season 2, with 7,682.6 particles per 20 g of sediment. The overall results from both seasons are summarized in Figure 10. The density of polymers directly influences the transport of plastic debris within the water column. Factors such as biofouling, entanglement with organic matter and debris, and buoyancy affect the density of polymers, leading to their sinking behavior (Andrady, 2017).

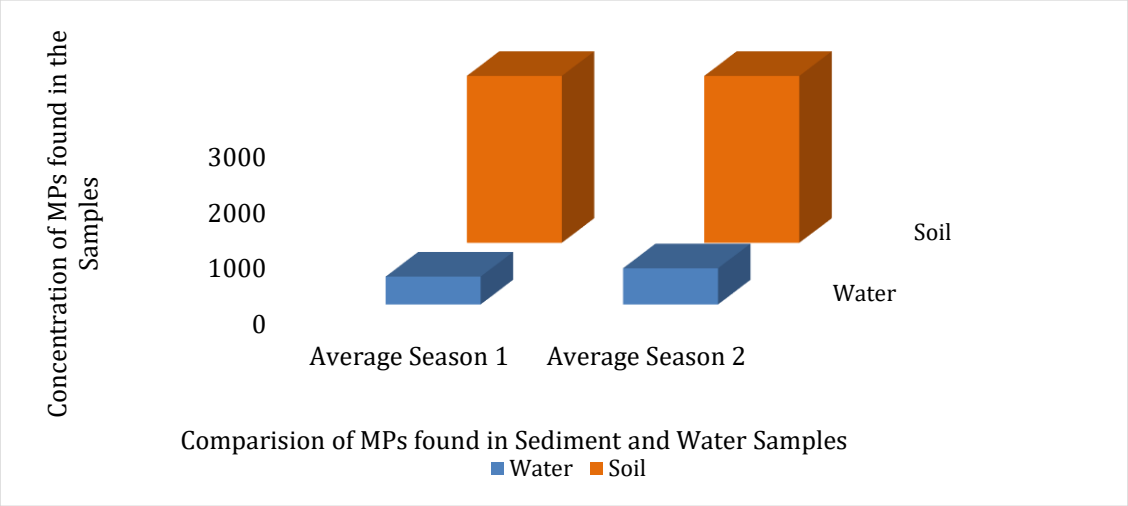


Figure 9. Summarizes the Difference between Seasons

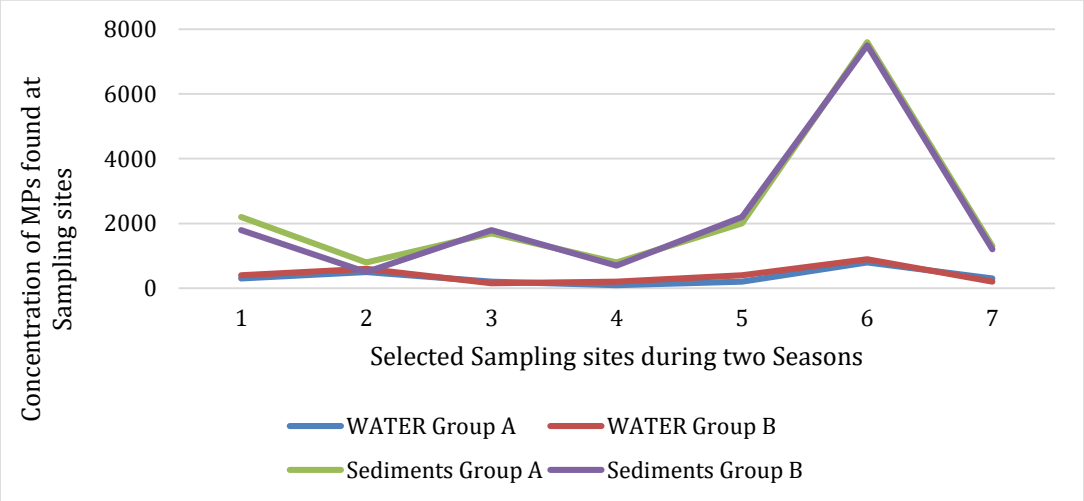


Figure 10. Difference in Concentrations of MPs between Two Seasons at 7 of the Sampling Sites

4.4 Two-Tailed Test for Water and Sediment Samples

An independent t-test comparing MP concentrations in water and sediment samples showed no significant seasonal differences. For sediments, Season 1 (M = 2,472.0, SD = 5,596.2) and Season 2 (M = 2,392.7, SD = 2,619.32) yielded (t [25] = 0.05, p = 0.95), while for water, Season 1 (M = 330, SD = 262.4) and Season 2 (M = 521.2, SD = 388.5) showed (t [25] = -0.9, p = 0.34). Both p-values exceed the alpha level (0.05), indicating no significant seasonal variation. However, comparisons between water and sediment samples showed significant differences in Season 1 (p = 0.04) and a near-significant result in Season 2 (p = 0.06). Higher MP concentrations, especially spheres and fragments, were found in sediments (p = 0.001) compared to water (p = 0.1).

Results of Characterization of Polymers Types Using Attenuated Total Reflectance Fourier Transformed Infrared Spectroscopy ATR-FTIR

Identifying polymer types is essential for understanding their sources, occurrence, distribution, and environmental fate. The FT-IR technique was developed to characterize and analyze polymeric materials [26]. A diverse array of polymeric particles was observed during the characterization phase. This method is the fastest and most practical way to identify polymer types without altering the sample's original nature. The spectra obtained through absorption were compared with an existing database to determine the polymer types (Löder et al., 2015). All spectra either matched directly or differed by no more than four readings from the existing database. The identified groups were organized according to the resin codes of the polymers. Figures 11 and 12 summarize the percentage concentrations of polymers found in both water and sediment samples.

4.5 Interpretation of ANOVA Results

A one-way ANOVA was performed to compare the concentrations of different plastic types in water and sediment samples across Season 1 and Season 2. Tukey's HSD Post Hoc analysis showed that water samples had a mean concentration of M = 2.21 (SD = 1.48), while sediment samples had a mean of M = 5.0 (SD = 4.2), indicating a significant difference. The coefficient of determination (R^2) was 0.812 for water samples and 0.76 for sediment samples, demonstrating a strong correlation in both cases.

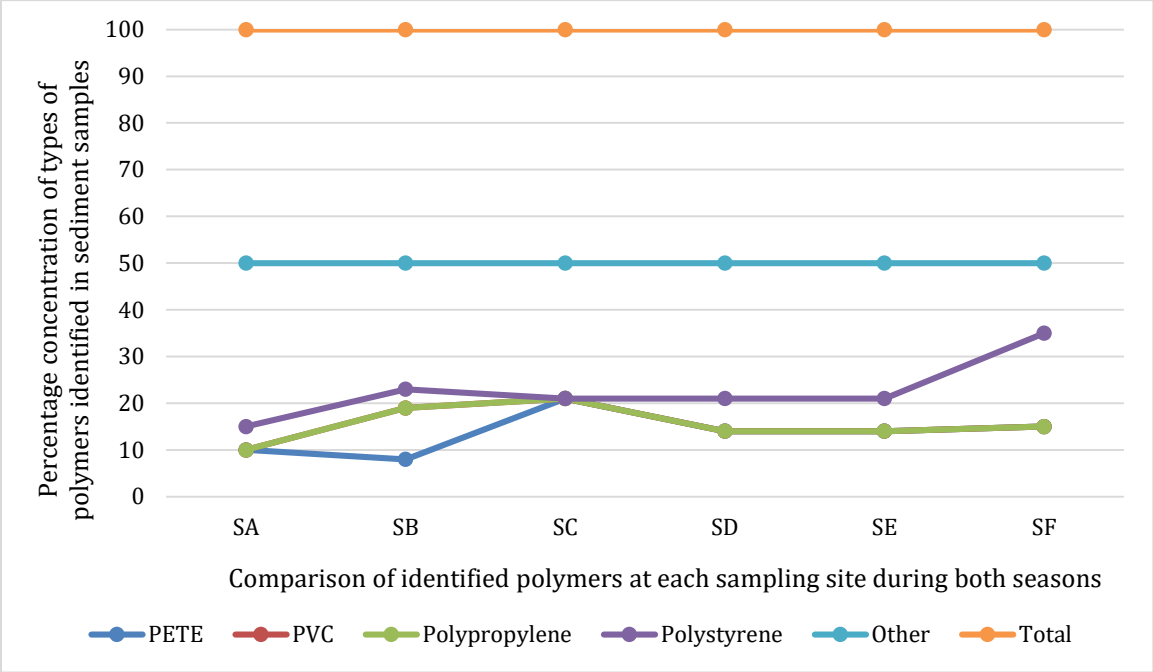


Figure 11. Percentage Concentration of Polymers Identified in Sediment Samples

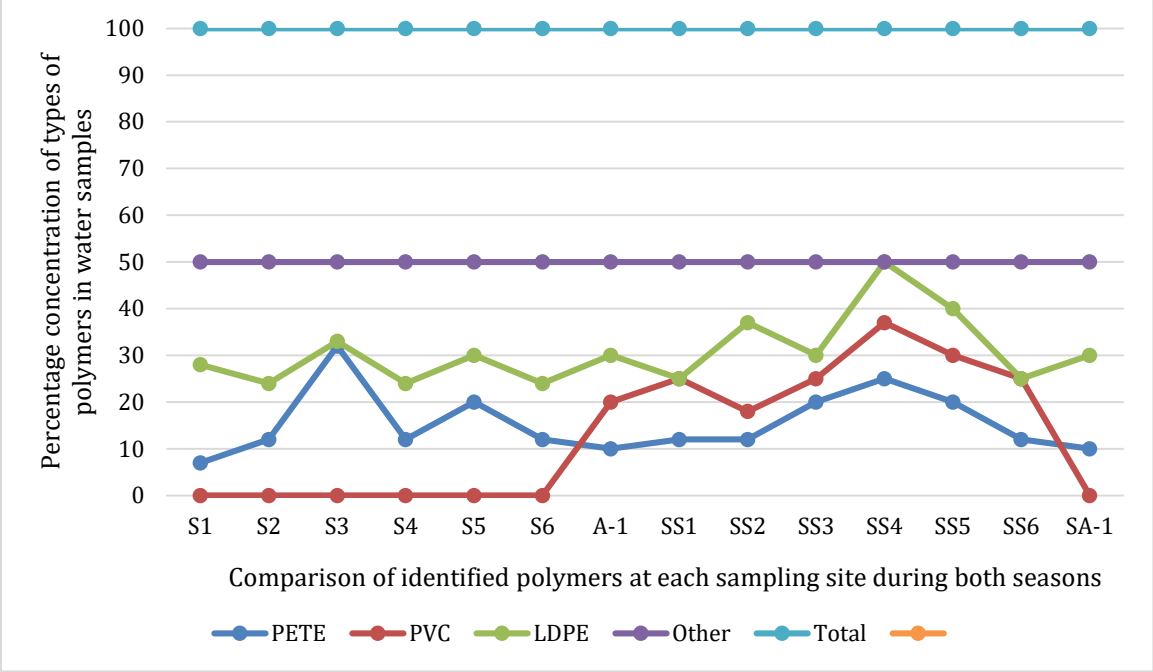


Figure 12. Percentage Concentration of Polymers Identified in Water Samples

Table 2 and Table 3 summarize the identified types of plastics in water and sediment samples, respectively. While "Other" plastics (Resin code 7) have the most significant impact, the presence of PP, PVC, PS, and PETE is also noteworthy, as reflected by the 95% confidence range (Figure 13, Figure 14).

Table 2. Model Parameters of MPs Found in Water Samples

Source	Value	Standard Error	T	Pr > |t|	Lower Bound (95%)	Upper Bound (95%)
Intercept	4.500	0.178	25.221	< 0.0001	4.142	4.858
Sample - LDPE	-3.500	0.425	-8.240	< 0.0001	-4.352	-2.648
Sample - Other	-2.654	0.257	-10.321	< 0.0001	-3.170	-2.138
Sample - PETE	-3.143	0.252	-12.455	< 0.0001	-3.649	-2.637
Sample -PVC	-3.192	0.257	-12.415	< 0.0001	-3.708	-2.676
Sample - Total	0.000	0.000				

Table 3. Model Parameters of MPs Found in Sediment Samples

Source	Value	Standard Error	T	Pr > |t|	Lower Bound (95%)	Upper Bound (95%)
Intercept	11.500	0.633	18.178	< 0.0001	10.227	12.773
Samples-Other	-5.955	0.915	-6.509	< 0.0001	-7.795	-4.114
Samples-PETE	-8.500	0.895	-9.501	< 0.0001	-10.300	-6.700

Source	Value	Standard Error	T	Pr > |t|	Lower Bound (95%)	Upper Bound (95%)
Samples-PVC	-9.500	1.167	-8.144	< 0.0001	-11.847	-7.153
Samples-Polypropylene	-10.500	1.674	-6.273	< 0.0001	-13.867	-7.133
Samples-Polystyrene	-9.500	0.915	-10.385	< 0.0001	-11.340	-7.660
Samples-Total	0.000	0.000				

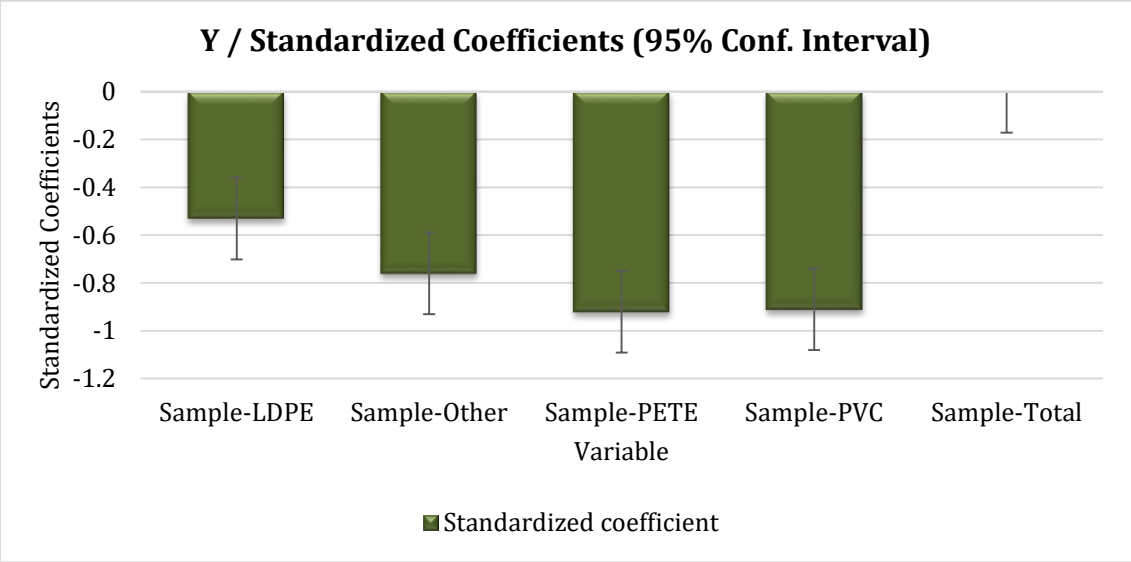


Figure 13. Standardized Regression Coefficients of Microplastic Abundance in Water Samples

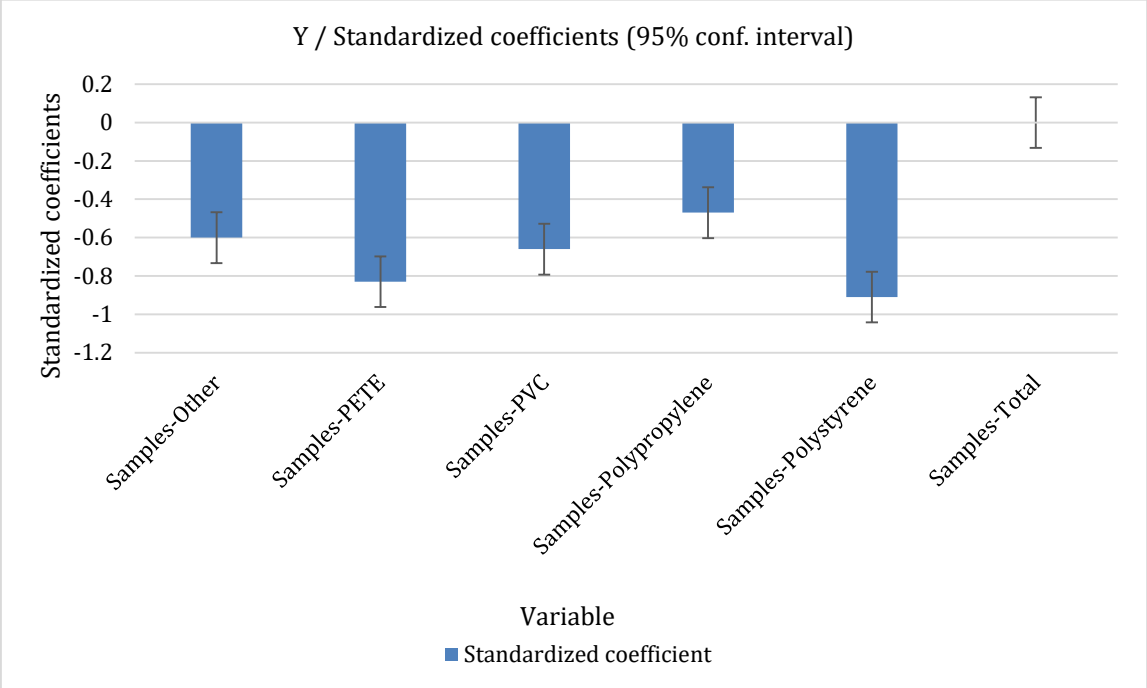


Figure 14. Standardized Regression Coefficients of Microplastic Abundance in Sediment Samples

4.6 Polymeric Compounds Identified in Water Samples

The ATR-FTIR analysis of water samples from both seasons identified various plastics, including Polyethylene Terephthalate (PETE) [27], Polyvinyl Chloride (PVCs) [28], and miscellaneous plastics (Resin code 7). In Season 1, only one sample contained Low-Density Polyethylene (LDPE), while two samples in Season 2 also had LDPE. Notably, no samples contained High-Density Polyethylene (HDPE), polypropylene (PP), or Polystyrene (PS).

The absorption spectra confirmed PETE with peaks at 434 cm^{-1}, 451 cm^{-1}, 455 cm^{-1}, 3421 cm^{-1}, and other similar ranges. PETE is commonly used in polyester, biodegradable plastics, bottles, and packaging. PVC derivatives were also detected with peaks at 420 cm^{-1} to 470 cm^{-1}, 1259 cm^{-1}, and 2063 cm^{-1} to 2094 cm^{-1}. LDPE was identified by a peak at 1651 cm^{-1}. Other plastics included Polyacrylic fibers (peaks at 461 cm^{-1}, 794 cm^{-1}, and 1018 cm^{-1}), Polyaniline (3452.7 cm^{-1}), Nylons (1633.7 cm^{-1}), Poly Methyl Methacrylate (PMMA) (1643 cm^{-1}), and Polycarbonate (1014 cm^{-1}).

4.7 Polymeric Compounds Identified in the Sediments

Sediment samples from various sites revealed a wide range of polymers. All samples contained Polyethylene Terephthalate (PETE) [27], Polystyrene (PS) [24], and miscellaneous plastics (Resin code 7). Polyvinyl Chloride (PVC) was found at one site in Season 1 and four sites in Season 2, while Polypropylene (PP) was detected at two sites in Season 2. Neither High-Density Polyethylene (HDPE) nor Low-Density Polyethylene (LDPE) was present in any sediment samples.

PETE was identified with absorption peaks at 1053 cm^{-1}, 1795 cm^{-1}, 2874 cm^{-1}, and others, appearing mostly in packaging, viscose, and fabric forms. PVC was detected with peaks at 1425 cm^{-1}, 1437 cm^{-1}, and 2926 cm^{-1}. PS was found with peaks at 532 cm^{-1}, 694 cm^{-1}, 1259 cm^{-1}, and 2962 cm^{-1}. PP was identified at 711 cm^{-1} and 1458 cm^{-1}. Additional plastics included Polyurethane (640 cm^{-1}, 2852 cm^{-1}), Biodegradable Polyurethane (1871 cm^{-1}), latex (1444 cm^{-1}), Polyacrylic Fiber, Acrylonitrile Butadiene Styrene (ABS), Poly Methyl Methacrylate (PMMA), Polycarbonate, Nylon 6, and other polymers. This diversity reflects the industrial and commercial activities affecting the river's environment.

5. CONCLUSION

The investigation of microplastic concentrations in the Soan River revealed significant findings. Water samples averaged 318 particles per 0.25 m^2 in the first season, rising to 500 particles in the second season. Sediment samples showed 2,466 and 2,341.7 particles per 20 g of sediment. The high presence of microplastics (0.2 mm) highlights their widespread infiltration, posing risks to food chains at all trophic levels. PET, PVC, and PS were the most common polymers found in both water and sediments. Establishing baseline data is essential for developing strategies to mitigate this environmental issue.

5.1 Recommendations

Based on this study's findings, several recommendations are made to tackle microplastic pollution. The widespread use of plastics in domestic and commercial settings requires a detailed analysis of their components to assess potential hazards. Each plastic type should be evaluated for its environmental impact and contribution to microplastic pollution. Expanding research to other environmental spheres—such as soils, atmospheric particles, and other potential reservoirs—is vital for a comprehensive understanding of microplastic contamination. Mapping microplastic hotspots in freshwater resources will help target critical areas for intervention and allocate resources more effectively. Additionally, incorporating microplastics into National Environmental Quality Standards is crucial for setting regulatory benchmarks and enforcing pollution reduction guidelines. Further research into the impact of microplastics on food chains is also essential to understand the risks to human health and ecosystems.

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Author Contribution Statement

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Nageen Sohail	✓			✓	✓				✓					
Abdullah Khalid		✓				✓	✓			✓				
Dania Sofia Rahim				✓				✓			✓			
Sofia Khalid	✓		✓									✓	✓	✓

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

No conflict of interest.

Informed Consent

Not Applicable.

Ethical Approval

Not Applicable.

Data Availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

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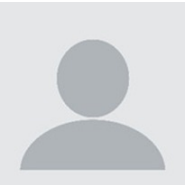
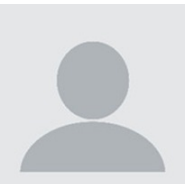
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