

Microplastic Contamination in Harike Wetland, a Ramsar Site in Punjab: An Environmental Assessment

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Abstract

Microplastics (MPs) are increasingly recognized as pervasive pollutants in aquatic ecosystems, posing significant threats to environmental health and food chain integrity. This study focuses on the presence, distribution and characteristics of MPs in water samples from the Harike wetland, a critical biodiversity hotspot in Punjab. Water samples were systematically collected and subjected to a comprehensive process involving filtration and subsequent analysis to isolate and identify MPs. Characterization of MPs was conducted based on their size, morphology and color, using advanced spectroscopic techniques including Fourier Transform Infrared (FTIR) spectroscopy and Pyrolysis-Gas Chromatography Mass Spectrometry (py-GCMS). These analyses revealed the presence of MPs, with polyethylene and polypropylene emerging as the dominant polymer types in the samples. Seasonal variations in MP concentrations were evident, with the highest levels recorded during the summer season.

Specifically, the dominant morphology of MPs was found to be fragments, indicating diverse sources and pathways of MP pollution within the wetland. The high levels of MPs present risks to aquatic life and ecosystem health, highlighting the need for better monitoring and mitigation strategies. This study also serves as a baseline for future research on the long-term ecological effects of MPs in aquatic environments.

Keywords: Wetland, microplastics, zooplanktons, food chain.

Introduction

The global increase in plastic production and use has led to the widespread dispersion of small plastic particles, known as microplastics (MPs), typically less than 5 millimeters in size. This surge in plastic consumption across various sectors has resulted in MPs being found in many ecosystems including terrestrial, freshwater and marine environments.

As a result, human activities such as plastic manufacturing, use and disposal have contributed to the extensive distribution and persistence of these tiny plastic fragments in natural habitats^{9,12}. The pervasive presence of plastic debris has raised significant concerns regarding the welfare of aquatic species with human activities, especially in urban

areas, being major contributors to the escalating pollution of the environment with plastic materials.

The extensive use of plastics and polymers has profoundly transformed daily life, leading to substantial changes in lifestyle and consumption patterns. In 2016, global plastic production reached an unprecedented 335 million tons, underscoring the scale of this environmental challenge. This surge in plastic production and consumption has resulted in the widespread dispersion of microplastics, particles typically smaller than 5 millimeters across various ecosystems including terrestrial, freshwater and marine environments¹³. The continuous release and accumulation of these minute plastic fragments are exacerbated by anthropogenic activities such as plastic manufacturing, usage and disposal practices, thereby contributing to their persistent presence in natural habitats and posing significant risks to wildlife and ecosystem health^{5,7}.

Over the past decade, the accumulation and presence of non-biodegradable plastic debris in the environment have emerged as a pressing global issue¹⁹. Consequently, the breakdown of plastics through physical, thermal, chemical and biological processes has resulted in microplastics, which are minuscule plastic fragments measuring approximately 5 mm in diameter³⁵. The reduced size of microplastics allows them to permeate aquatic organisms more readily, thereby increasing their likelihood of entering the food chain. Due to their microscopic dimensions, resembling natural food particles and their pervasive presence, aquatic species are more prone to ingest microplastics²⁵.

The discharge of treated effluents originating from water and wastewater treatment facilities (WWTPs) represents a substantial and noteworthy source contributing to the prevalence of microplastics (MPs) within the environmental milieu. The intricate network of WWTPs serves as a pivotal conduit through which various anthropogenic contaminants, including MPs, are introduced into aquatic ecosystems²⁶. Microplastic pollution has emerged as a pressing environmental concern globally, with its detrimental impacts extending to diverse ecosystems including wetlands.

The Harike Wetland, located at the confluence of the Beas and Sutlej rivers in the Indian state of Punjab, represents a critical habitat supporting rich biodiversity and providing essential ecosystem services¹⁷. Microplastics act as substrates that adsorb diverse persistent organic contaminants (POPs), potentially causing harm to the environment as they release specific pollutants³⁴. The United States Environmental Protection Agency (USEPA) has

identified chemical additives, pesticides, organic pollutants and colours found in plastics as priority pollutants, along with heavy metals and persistent, bioaccumulative and harmful (PBT) substances¹⁸. Providing a concise overview of the worldwide issue of plastic pollution, annually, an estimated 5 trillion plastic bags are utilized globally. Furthermore, around 13 million tons of plastic debris infiltrate our oceans and seas on a yearly basis.

Moreover, the production and use of plastic in the recent decade has surpassed that of the entire preceding century. Furthermore, almost 50% of the plastic we utilize is specifically designed for one-time use or throwaway applications and plastic garbage constitutes more than 10% of the overall daily waste produced¹⁶. Zooplankton, found in various aquatic environments worldwide, including contaminated and municipal wastewater, are organisms affected by this issue. Zooplankton plays a crucial role in aquatic ecosystems, contributing to the food chain, food webs, energy flow and nutrient cycling, thereby playing a significant ecological role in all ecosystem elements³¹.

Zooplankton refers to the organisms occupying the lower trophic level of the aquatic food web. Nevertheless, the increasing abundance of MPs in freshwater ecosystems has generated apprehension regarding the possible ecological disturbances resulting from the consumption of these particles by zooplankton and other animals. The presence of microplastic contamination poses a severe threat to zooplankton. Previous studies have shown that zooplankton consuming microplastics can accumulate 2-7 particles per day in juvenile salmon in coastal British Columbia⁷.

This research study aims to investigate the variability of microplastic contamination over time and across different spatial scales within the water column and freshwater crustaceans inhabiting the wetland. By integrating comprehensive field surveys, laboratory analyses and spatial modeling techniques, this study endeavors to provide insights into the sources, distribution patterns and potential ecological consequences of microplastic pollution in this crucial freshwater ecosystem.

Material and Methods

Sampling Area: Harike wetland is among the largest freshwater wetlands located in the northern region of India. Situated at the merging point of two perennial rivers, Beas and Sutlej, the Harike Wetland Wildlife Sanctuary encompasses this unique ecosystem. Formed through human intervention, this freshwater wetland possesses characteristics of riverine and lacustrine environments. Located within the Indian State of Punjab, Harike wetland has garnered international recognition as a significant wetland area³⁰. The Harike wetland ecosystem, spanning an expansive area of 285.1 square kilometres, extends across four districts within the Indian State of Punjab, namely Amritsar, Firozpur, Kapurthala and Jalandhar. Positioned between coordinates 31.1700° N and 75.2000° E, the

wetland is nourished by the Satluj and Beas rivers along with their tributaries. Originating from the lofty Himalayas, these perennial rivers traverse vast distances before coursing through the State of Punjab. However, the wetland faces degradation primarily due to the contamination of the Sutlej river which receives industrial effluents originating from Ludhiana¹⁴.

The Harike wetland attained Ramsar site designation in 1990, as documented by the Ramsar Convention of 2008. Subsequently, the State Government of Punjab declared it a bird sanctuary under the name "Harike Wetland Bird Sanctuary" in 1992, initially allowing fishing activities. However, with the enactment of the Wildlife Act in 2000, fishing activities were completely prohibited within the marsh area²⁰. In Punjab, the summer period (April to June) is characterized by elevated temperatures reaching up to 45 °C and dry, hot air. The monsoon season (July to September) is distinguished by milder temperatures and varying degrees of rainfall, ranging from average to heavy. During the winter months (November to January), daytime temperatures typically range between 10 and 15 °C, with overnight lows dropping to 0 °C, according to the Indian Meteorological Department.

Punjab receives annual rainfall ranging from 532 mm in the plains to 890 mm in the northern sub-mountainous regions, with precipitation decreasing from north to south²⁷. In this study, the three sampling sites have been taken. The first sampling site is the Indira Gandhi Canal. The Indira Gandhi canal connects Rajasthan and Punjab and is 650 kilometres long. The Indira Gandhi canal features a water barrage system that discharges 18000 cubic meters of water per day¹. Because the water from this canal is used for various reasons in Rajasthan such as agriculture and drinking, it is critical to understand the MP pollution in water.

The second sampling site, Gurudwara Nanaksar Sahib, was selected due to its association with anthropogenic activities. The third sampling site is the bird sanctuary. Since migrating birds' arrival and departure affect the water quality³², it is vital to use the bird sanctuary as one of the sampling sites to determine how the physicochemical parameters of the wetland change throughout the year.

Water Sample Collection: Sampling was conducted during specific temporal intervals in April, August, November 2022 and February 2023, within the time frame of 7:00 to 9:00 am. The seasonal delineation adheres to the classification system of the Indian Meteorological Department in New Delhi, comprising of summer, monsoon, post-monsoon and winter seasons. Surface water samples were acquired using the drag sampling method, employing a student plankton net with a mesh size of 50 micrometres attached to a BOD bottle and deployed at a depth of 15 cm.

Prior to sampling, meticulous prewashing of the equipment with detergent was carried out. Sampling was conducted

from three distinct sites within the study area, facilitated by a boat for access. On-site filtration of water samples was performed at each sampling location. The collected net was carefully packaged to ensure sample preservation during transportation to the laboratory for subsequent processing and analysis. Sampling from each of the three sites yielded three discrete samples.

MPs Extraction from Water by Density Separation and purification: Utilizing a student plankton net featuring a mesh size of 50 micrometers and deployed to a depth of 15 cm at the sampling site, water samples were subjected to on-site filtration. Subsequently, the net underwent careful packaging for transportation to the laboratory, where further processing occurred. In the laboratory setting, a thorough cleaning of the entire net and particle collection was achieved through rinsing with 2.5 liters of distilled water. Pre-screening is conducted to eliminate large particles of 5 mm to 1 mm in size¹⁰.

Employing forceps, potentially discernible particles were meticulously segregated for closer examination. The particles underwent a hot needle test to ascertain their plastic nature. Subsequently, their sizes were recorded both before and after the test. Particles measuring 5mm or less were classified as microplastics, while those exceeding 5mm were excluded from this classification. Post-test, shrunken particles were selected for analysis to determine polymer composition via Fourier Transform Infrared (FTIR)

spectroscopy⁶. The residual wash-off underwent filtration using a series of sieves with mesh sizes ranging from 400 μm to 60 μm , aimed at capturing potential microplastic particles across various size ranges. Subsequent to this sieving process, the filtrate underwent further filtration using smaller mesh sizes. The residue deposited on the filter cloth was then rinsed with distilled water per mesh size from the cloth for each of the four mesh sizes.

After undergoing organic digestion and density-based separation to eliminate organic matter, plastic particles were further processed through density gradient separation²⁴. 4 ml of 30% H_2O_2 were added to each 10 ml sample to remove organic materials completely. To facilitate the flotation of potential microplastic particles on the wash-off, a sodium iodide solution with a density of 1.8 g/cm^3 was introduced, establishing a density gradient. Following the density gradient procedure, the upper layer of the sample was subjected to centrifugation at 3500 rpm for 15 minutes. The resulting supernatant, presumed to contain plastic particles due to their low density, was collected.

To verify the presence and type of plastic in the remaining sample, it was weighed and evenly divided for analysis using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Pyrolysis-Gas Chromatography-Mass Spectrometry (Pyr-GC-MS), following the methodology outlined by Nuelle et al²⁴.

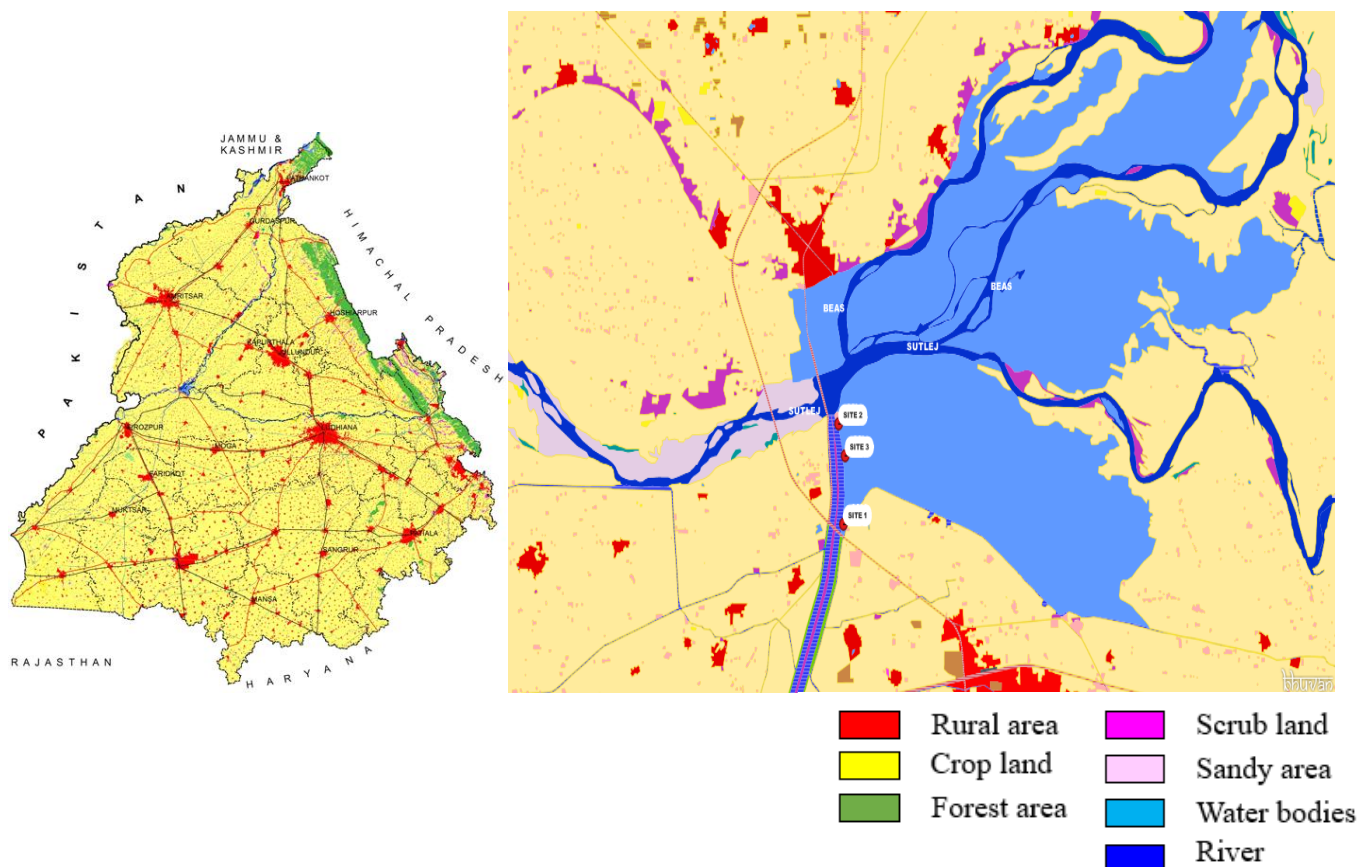


Fig. 1: The map of Punjab and map of Harike wetland showing the three different sites.

Visual Analysis: Before acquiring spectral data, detailed documentation of visual characteristics such as color, morphology and size was conducted for each particle. Morphological categorization distinguished particles into beads, fragments, or films.

Size measurements were performed using MICAPS software in conjunction with a stereo microscope, with reported dimensions based on the length of the longest axis of the particle.

Chemical Analysis: The samples were subjected to FTIR. Fourier transform infrared (FTIR) spectroscopy scans samples and analyses their chemical characteristics using infrared light. FTIR identifies functional groups in chemicals in a sample. Each residue was analysed using FTIR. To avoid interference from water vapour peaks during FTIR measurement, the sample was dried in an oven at 55°C for 10 minutes. Then Py GC-MS of sample was done to confirm the microplastic type present. GC-MS combines gas chromatography and mass spectrometry to identify chemicals in a sample by separating them using GC and comparing their mass to a standard. These are the conditions to be followed for carrying out Py-GCMS. [Type of sample: Solid state, Sample Storage and toxicity details: stored in glass vials. Column Details-Column Flow rate- 0.25mm, length 40m, df 0.25 µm, Column Oven Temperature- 40°C- 32 °C (20 °C/min, 1min hold), GC Temperature Program-40 °C to 180 °C at 15 °C/min and then to 300 °C at 5 °C/min, held for 12 min, Detector Temperature (MS)- 230 °C, Interference, Temperature- 300 °C, Pyrolyser Details-Single Shot Analysis].

Results

Physical Properties and Abundance of Microplastics in the Harike Wetland: Microplastics in the Harike wetland exhibit diverse physical properties including variations in size, shape, color and polymer composition. These particles typically range from 1 micrometer to 5 millimeters in diameter, with the most common shapes being fragments and fibers. Factors such as water currents, agricultural runoff and nearby urban activities contribute to the accumulation of microplastics in the ecosystem.

Additionally, the color of microplastics varies widely with common hues including blue, green and black, which can affect their visibility and impact on aquatic organisms. Understanding the physical properties and abundance of microplastics in the Harike wetland is crucial for assessing their ecological implications and developing effective mitigation strategies.

The figure 2 shows the visually collected plastic particles confirmed by the hot needle test across different sites and seasons. These particles were subsequently sent for FTIR analysis to determine the nature of the plastic. In summer, site 1 and site 3 each recorded 5 plastic particles, while site 2 had 4, totaling 14 particles across all sites. During the

Monsoon, the total number of particles decreased to 9, with 2 particles each at sites 1 and 2 and 4 particles at Site 3. Post-monsoon, site 1 again had 5 particles, site 2 had 1 and site 3 had 4, making a total of 10 particles.

In winter, site 1 maintained a consistent count of 5 particles, while site 2 and site 3 had 3 particles each, leading to a total of 11 particles. This data highlights the seasonal and site-specific variation in the occurrence of plastic particles.

Shape and Size: Five categories were established for the morphologies of MPs that were filtered out of water samples: foam, fragment, sphere, film and fiber. Fibers and fragments were found to be the most common types of plastic particles in the Harike wetland at all three sampling locations. This result was similar with those of previous studies on freshwater systems³⁶. The increase in plastic use across various sectors has resulted in the presence of microplastics (MPs) in diverse ecosystems. These plastics break down into smaller particles through photolytic degradation and eventually enter water bodies, impacting terrestrial, freshwater and marine environments.

Consequently, human activities like plastic production, utilization and disposal have significantly contributed to the widespread distribution and persistence of these tiny plastic fragments in natural ecosystems⁹.

Plastic fibers are widely used, that is why they make up a significant fraction of research, particularly in freshwater environments¹⁸. Due to its proximity to human activity, the Harike wetland was able to receive a lot of fiber. The health of the wetlands is at risk due to the high population density of informal settlements, which puts additional strain on the waste disposal systems²³. The wetland is made by the confluence of two main rivers Sutlej and Beas which pass through cities, villages, farmlands and factories. Along the way, household sewage, wastewater treatment plant discharge, surface and underground runoff, agricultural cultivation and atmospheric migration get mixed¹⁵.

In the Harike wetland in Punjab, microplastics are predominantly observed in a range of sizes, reflecting the diverse nature of plastic pollution in this ecosystem. First we collected the visually suspected. These particles vary from smaller than 1 mm to approximately 5 mm in diameter. The smaller microplastics, often less than 1 millimeter, are particularly of concern due to their ability to be ingested by a wide range of aquatic organisms including those at the base of the food chain.

Larger particles, while less likely to be consumed directly, can still break down into smaller fragments over time, further contributing to the pollution load. The distribution of microplastic sizes in Harike wetland highlights the complexity of plastic contamination and underscores the need for comprehensive monitoring and management efforts to address this environmental issue effectively.

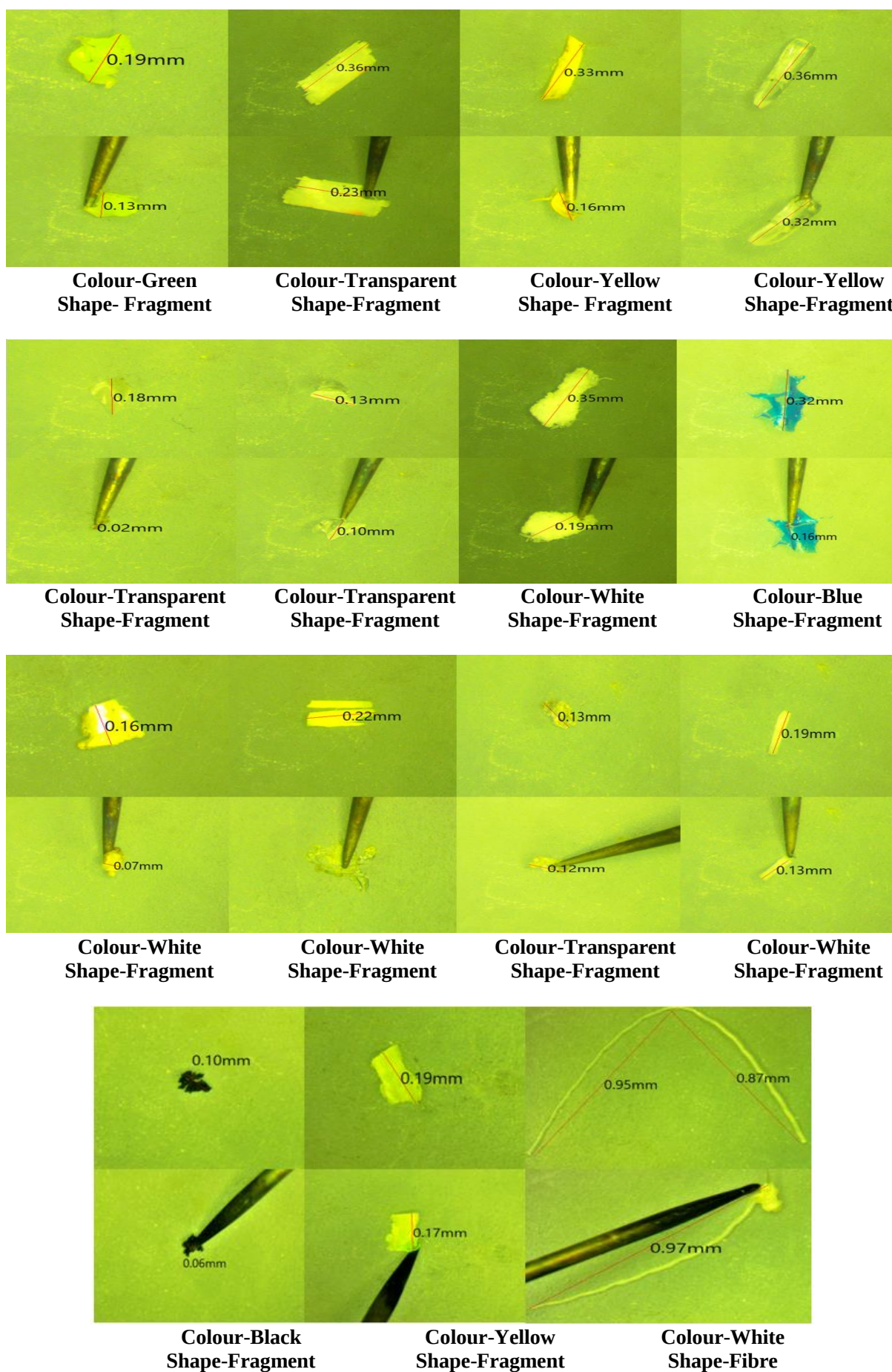


Fig. 2: Illustrating the appearance of microplastic (MP) particles before and after undergoing the hot needle test

Colour Abundance of MPs found in Harike Wetland:

The observed microplastics (MPs) particles displayed a range of colors including white, black, transparent, blue, green, yellow, brown and red. The principal determinant influencing the color spectrum observed in microplastics (MPs) predominantly originates from the assortment of plastic constituents, which undergo contamination primarily through urban effluent discharge³⁹. The presence of colored synthetic fibers from manufactured garments further diversifies MP coloration dynamics. Upon degradation, these fibers release pigment residues, leading to the emergence of red or pink-tinted MPs within aquatic ecosystems.

Thus, the interplay of contamination sources, environmental conditions and material properties intricately shapes the color profile of microplastic pollutants in natural settings²¹. Black MPs were discovered in 16.67% of samples at sites 1 and 3 during the summer. Site 2 (33.33%) and site 3 (33.33%) had the highest concentration of white MPs, whilst site 1 (33.33%) and site 2 (33.33%) had the highest concentration of transparent MPs. There were only blue MPs observed at site 1 (16.67%). At site 3, green and yellow MPs were detected in 16.67% of the samples. Black MPs were only observed at site 3 (16.67%) during the monsoon, but transparent MPs were evenly distributed (16.67%) among all sites. The blue members of parliament were not present.

Black MPs were only found at site 1 (16.67%) after the rain. At Sites 1 (16.67%) and 3, (33.33%), white and transparent MPs were noted, but blue MPs were more prevalent at sites 2 and 3.

During the winter, black MPs (16.67%) and white MPs (16.67%) were observed at sites 1 and 3. Brown MPs were at site 2 whereas green and yellow MPs were at site 1. Transparent MPs were consistently present at all sites (16.67%). Harike wetland is surrounded by large agricultural province in which plastic products are used frequently in agricultural cultivation processes, such as for films and drip irrigation belts, which was a potential reason for the diversity of microplastic colours^{3,33}.

The table 1 compares the weight of sample residues collected from three different sites across four seasons using various mesh size filter cloths. During the summer, site III had the highest residue weight at 50.8 ± 0.7 mg, followed by site I at 39.6 ± 0.4 mg and site II at 37.5 ± 0.5 mg. In the Monsoon season, residues decreased, with Site III still leading at 37.3 ± 1.1 mg, while sites I and II had similar values of 31.3 ± 0.9 mg and 31.6 ± 0.57 mg respectively. Post-Monsoon, the residue weight was highest at site I with 19.6 ± 0.4 mg, compared to Site III at 17.3 ± 0.2 mg and site II at 11.8 ± 0.7 mg.

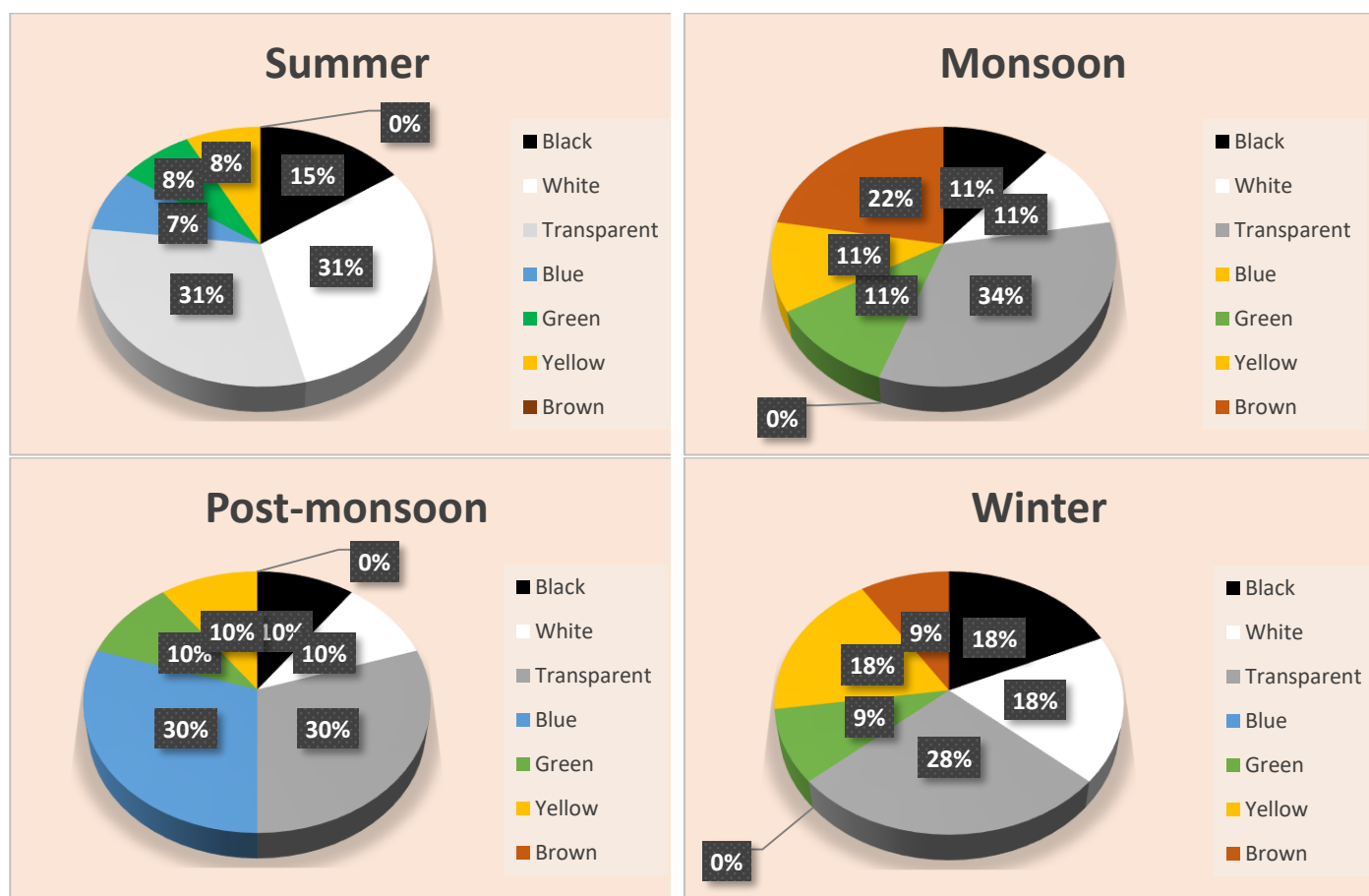


Fig. 3: Microplastic color abundance in the water found in the harike wetland in four different seasons: summer, monsoon, post- monsoon and winter

In winter, site I again recorded the highest residue weight at 30.33 ± 0.4 mg, with site III at 21.6 ± 2 mg and site II at 14.6 ± 0.2 mg. This data underscores the seasonal and site-specific variations in residue accumulation across the three sites.

Characterization of Plastics Particles by Fourier Transform Infrared Spectroscopic Analysis: Fourier Transform Infrared (FT-IR) spectroscopy employs infrared radiation to irradiate polymer particles and subsequently analyses the wavelengths reflected from these particles to determine their composition. Using the Cary 630 FT-IR spectrophotometer, the presence of PS in foam spheres sized 3–5 mm (Figure) and PE in fragments smaller than 5 mm (Figure) was confirmed, which aligns with findings similar to Mariano et al²². The Fourier-transform infrared (FTIR) analysis of the suspected microplastic particle collected from site 2 identifies it as polystyrene (Fig. 4). The FTIR spectrum provides detailed information on the functional groups present in the sample, correlating with specific peaks observed in the infrared range.

A peak at 3291 cm^{-1} corresponds to an aromatic C-H stretch. C-H stretching is also observed at 2914 cm^{-1} and 2849 cm^{-1} ,

indicative of alkyl group presence. The aromatic ring stretch is represented by peaks at 1632 cm^{-1} and 1452 cm^{-1} , signifying vibrational modes of the aromatic ring. Additionally, CH_3 bending is detected at 1029 cm^{-1} . The spectrum further reveals an aromatic C-H out-of-plane bend at 695 cm^{-1} and an aromatic ring out-of-plane bend at 537 cm^{-1} , demonstrating the complex structural features of the aromatic system inherent to polystyrene.

Similarly, the FTIR analysis of particles collected from site-1 confirms the presence of polypropylene (Fig. 5). The spectrum delineates several characteristic functional groups. A peak at 2954 cm^{-1} corresponds to an aromatic C-H stretch, indicative of aromatic hydrocarbon structures. C-H stretching is observed at 2914 cm^{-1} and 2876 cm^{-1} , suggesting the presence of alkyl groups within the polymer. Aromatic ring stretches are detected at 1493 cm^{-1} and 1452 cm^{-1} which correspond to the vibrational modes of the aromatic ring structure. Additionally, CH_3 bending is observed at 1029 cm^{-1} . The spectrum further reveals an aromatic C-H out-of-plane bending mode at 695 cm^{-1} and an aromatic ring out-of-plane bending mode at 537 cm^{-1} , illustrating the structural intricacies of the aromatic system within polypropylene.

Table 1

Comparative table for both sites and all seasons for depicting the weight of sample residues (mg) obtained from different mesh size filter cloth

Season	Site I mg/l	Site II mg/l	Site III mg/l
Summer	39.6 ± 0.4	37.5 ± 0.5	50.8 ± 0.7
Monsoon	31.3 ± 0.9	31.6 ± 0.57	37.3 ± 1.1
Post Monsoon	19.6 ± 0.4	11.8 ± 0.7	17.3 ± 0.2
Winter	30.33 ± 0.4	14.6 ± 0.2	21.6 ± 2

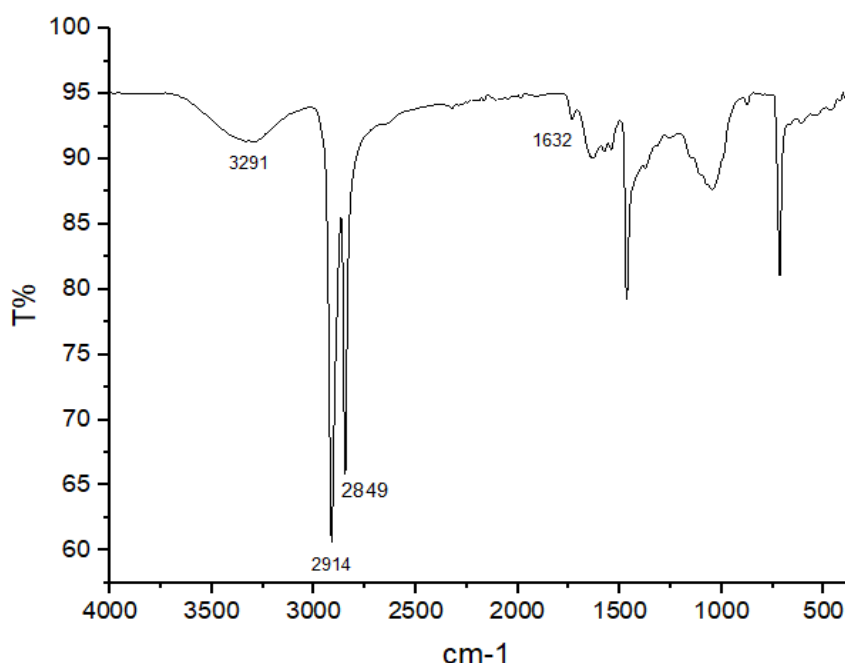


Fig. 4: FTIR spectra of particles confirmed as polystyrene collected from Site-2

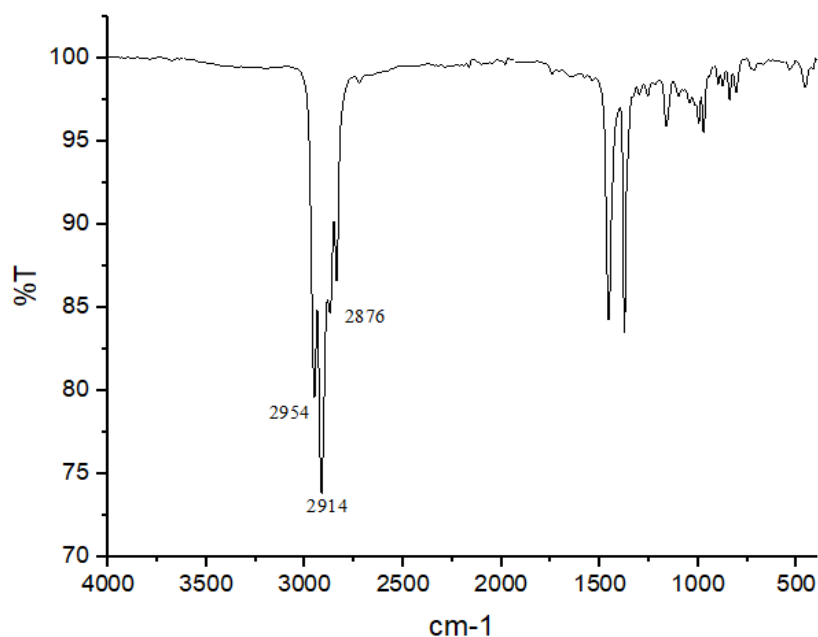


Fig. 5: FTIR spectra of particles confirmed as polypropylene collected from Site-1

Table 2

Showing the Characteristic pyrolyzate compounds of the 4 target plastic polymers, respective indicator ions and retention time under the defined Pyr-GC/MS conditions

Polymer	Pyrolyzate compounds	Indicator ion (m/z)	tR (min)
PET	Benzene Benzoic acid Diphenyl	123 156	26.510 to 42.230
PS	Benzene, 1,1'-(3-methyl-1-propene-1,3-diyl)bis	105 131	13.693
PE	1,20-Heneicosadiene (C ₂₁) Dodecene (C ₁₂)	83	15.1
PP	2,4-Dimethyl-1-heptene 2,4,6-Trimethyl-1-nonene	71	8.76

Characterization of Plastics Particles by pyr-GCMS:

Different particles collected from various sites, along with microplastic residues extracted using the density separation method, were analyzed by pyrolysis gas chromatography-mass spectrometry (pyr-GCMS). The pyr-GCMS data for these microplastic residues reveal a polymer with substantial hydrocarbon content. The observed profile includes long-chain alkanes, alcohols and esters²⁹. A polymer that aligns with this profile is polyethylene (PE), characterized by long chains of ethylene which corresponds well with the aliphatic hydrocarbon profile seen in the analysis. Specifically, compounds such as 1-Decanol and 2-Hexyl- are recurrently identified. Additionally, several peaks related to cyclohexane derivatives such as 1,2,3,5-tetraisopropyl-cyclohexane, were detected.

A suspected microplastic particle from site 1 displays a profile similar to polyethylene but with the notable presence of methyl groups branching off the main carbon chain. This branching indicates that the data is indicative of long-chain hydrocarbons typical of polypropylene pyrolysis products. In the analysis of various polymer types through pyrolysis, specific pyrolyzate compounds and indicator ions have been identified, each corresponding to different retention times (tR). For polyethylene terephthalate (PET), the notable pyrolyzate compounds include benzene, benzoic acid and diphenyl, with indicator ions at m/z 123 and m/z 156,

detected between 26.510 and 42.230 minutes. Polystyrene (PS) produces benzene, 1,1'-(3-methyl-1-propene-1,3-diyl)bis as a pyrolyzate compound, with indicator ions at m/z 105 and m/z 131, observed at a retention time of 13.693 minutes.

For polyethylene (PE), the pyrolysis yields 1,20-heneicosadiene (C₂₁) and dodecene (C₁₂), with a characteristic indicator ion at m/z 83, appearing at 15.1 minutes. Finally, polypropylene (PP) is characterized by the pyrolyzate compounds 2,4-dimethyl-1-heptene and 2,4,6-trimethyl-1-nonene, with an indicator ion at m/z 71 and a retention time of 8.76 minutes.

Discussion

This study highlights the occurrence of microplastics (MPs) in the Harike wetland, emphasizing increasing concerns about the ecological health of aquatic systems. The dominance of polyethylene (PE) and polypropylene (PP) among the identified polymers is consistent with global findings which attribute their prevalence in aquatic environments to their extensive application in consumer goods and industrial processes⁴. The prevalence of fragmented morphologies reinforces the notion that secondary MPs, formed through the degradation of larger plastic materials, represent a major source of pollution in this wetland¹¹.

Seasonal fluctuations in MP concentrations, with the highest levels recorded during summer, can be linked to several factors including intensified human activities, accelerated plastic breakdown due to elevated temperatures and diminished water flow in the dry season, leading to pollutant accumulation¹¹. These patterns underscore the critical importance of integrating temporal dynamics into the design of monitoring frameworks and mitigation strategies for MP pollution. Furthermore, this study demonstrates the efficacy of advanced spectroscopic techniques including FTIR and py-GCMS, in the precise characterization of MPs. These methodologies facilitated not only the identification of polymer types but also the determination of their probable sources. Notably, the predominance of PE and PP in the samples is likely attributable to agricultural runoff, domestic waste streams and inadequacies in plastic waste management within the region. Similar observations across other freshwater ecosystems reinforce the global prevalence and significance of this issue². The ecological consequences of MP pollution in the Harike wetland are significant, as MPs can serve as carriers for toxic chemicals and pathogens, amplifying their harmful effects on aquatic organisms²⁸.

The consumption of MPs by aquatic organisms has the potential to disrupt food web dynamics and adversely impact biodiversity. Additionally, the bioaccumulation and trophic transfer of MPs present significant risks to higher trophic levels including humans, through the ingestion of contaminated water or fish³⁷. Future research should aim to systematically quantify the long-term ecological and health risks associated with MP contamination in wetland ecosystems, while evaluating the potential of bioremediation and other advanced remediation strategies for MP removal. Additionally, extending analytical efforts to encompass sediment and biota samples would enable a more comprehensive and nuanced understanding of MP distribution, pathways and interactions within the wetland ecosystem.

Conclusion

This study on the Harike wetland reveals significant insights into the distribution and characteristics of microplastics (MPs) within this crucial aquatic ecosystem. Findings indicate substantial spatial and temporal variability in MP pollution, influenced by water flow patterns, seasonal changes and human activities. Predominant polymer types identified include polyethylene and polypropylene with fragments and beads suggesting sources such as urban runoff, plastic waste disposal and agricultural activities. The varying MP concentrations across different sampling locations underscore the complexity of MP pollution dynamics. These results emphasize the urgent need for continuous monitoring and targeted mitigation strategies to address specific sources and hotspots of MP contamination.

The study provides a crucial baseline for future research and policy initiatives aimed at managing and reducing MP pollution in the Harike wetland and similar environments.

Addressing MP pollution challenges requires a multifaceted approach involving scientific research, public awareness and policy intervention to protect aquatic ecosystem health and biodiversity.

References

1. Bhakar R., Analysis of hydrogeological system and land cover for assessment of risks to irrigated agriculture in Thar desert, Charanwala system of the Indira Gandhi canal project (2007)
2. Bläsing M. and Amelung W., Plastics in soil: Analytical methods and possible sources, *Science of the Total Environment*, **612**, 422–435 (2018)
3. Browne M.A., Galloway T.S. and Thompson R.C., Spatial patterns of plastic debris along estuarine shorelines, *Environmental Science & Technology*, **44**(9), 3404–3409 (2010)
4. Chen G., Feng Q. and Wang J., Mini-review of microplastics in the atmosphere and their risks to humans, *Science of the Total Environment*, **703**, 135504 (2020)
5. Colomer J., Müller M.F., Barcelona A. and Serra T., Mediated food and hydrodynamics on the ingestion of microplastics by *Daphnia magna*, *Environmental Pollution*, **251**, 434–441 (2019)
6. De Witte B., Catarino A.I., Vandecasteele L., Dekimpe M., Meyers N., Deloof D., Pint S., Hostens K., Everaert G. and Torreele E., Feasibility study on biomonitoring of microplastics in fish gastrointestinal tracts, *Frontiers in Marine Science*, **8**, 794636 (2022)
7. Desforges J.P.W., Galbraith M. and Ross P.S., Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean, *Archives of Environmental Contamination and Toxicology*, **69**, 320–330 (2015b)
8. Dilsha Davis and Narasimhan S., Detrimental Effects of Lithium on in vitro Seedlings of Pea (*Vigna radiata*), *Res. J. Biotech.*, **19**(1), 87-90 (2025)
9. Dris R., Gasperi J., Rocher V., Saad M., Renault N. and Tassin B., Microplastic contamination in an urban area: A case study in Greater Paris, *Environmental Chemistry*, **12**(5), 592–599 (2015)
10. Enders K., Lenz R., Stedmon C.A. and Nielsen T.G., Abundance, size and polymer composition of marine microplastics $\geq 10 \mu\text{m}$ in the Atlantic Ocean and their modelled vertical distribution, *Marine Pollution Bulletin*, **100**(1), 70–81 (2015)
11. Ermis H., Collins C., Saha S.K. and Murray P., Beyond Visibility: Microorganisms for tackling plastic and microplastic problems for cleaner future, *Chemical Engineering Journal*, **497**, 154585 (2024)
12. Geyer R., Jambeck J.R. and Law K.L., Production, use and fate of all plastics ever made, *Science Advances*, **3**(7), e1700782 (2017)
13. Issac M.N. and Kandasubramanian B., Effect of microplastics in water and aquatic systems, *Environmental Science and Pollution Research*, **28**, 19544–19562 (2021)

14. Kaur J., Chaudhary A., Kaur R. and Arora S., Assessment of mutagenic, genotoxic and cytotoxic potential of water samples of Harike wetland: A Ramsar site in India using different ex vivo biological systems, *Ecotoxicology*, **23**, 967–977 (2014)
15. Kumar A., Singh C.K., Bostick B., Nghiem A., Mailloux B. and van Geen A., Regulation of groundwater arsenic concentrations in the Ravi, Beas and Sutlej floodplains of Punjab, India, *Geochimica et Cosmochimica Acta*, **276**, 384–403 (2020)
16. Laskar N. and Kumar U., Plastics and microplastics: A threat to environment, *Environmental Technology & Innovation*, **14**, 100352 (2019)
17. Li J., Liu H. and Chen J.P., Microplastics in freshwater systems: A review on occurrence, environmental effects and methods for microplastics detection, *Water Research*, **137**, 362–374 (2018)
18. Lithner D., Larsson Å. and Dave G., Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition, *Science of the Total Environment*, **409**(18), 3309–3324 (2011)
19. Lönnstedt O.M. and Eklöv P., Environmentally relevant concentrations of microplastic particles influence larval fish ecology, *Science*, **352**(6290), 1213–1216 (2016)
20. Mabwoga S.O. and Thukral A.K., Characterization of change in the Harike wetland, a Ramsar site in India, using landsat satellite data, *SpringerPlus*, **3**, 1–11 (2014)
21. Maheswaran B., Karmegam N., Al-Ansari M., Subbaiya R., Al-Humaid L., Raj J.S. and Govarthanan M., Assessment, characterization and quantification of microplastics from river sediments, *Chemosphere*, **298**, 134268 (2022)
22. Mariano S., Tacconi S., Fidaleo M., Rossi M. and Dini L., Micro and nanoplastics identification: Classic methods and innovative detection techniques, *Frontiers in Toxicology*, **3**, 636640 (2021)
23. Migwi F.K., Ogunah J.A. and Kiratu J.M., Occurrence and spatial distribution of microplastics in the surface waters of Lake Naivasha, Kenya, *Environmental Toxicology and Chemistry*, **39**(4), 765–774 (2020)
24. Nuelle M.T., Dekiff J.H., Remy D. and Fries E., A new analytical approach for monitoring microplastics in marine sediments, *Environmental Pollution*, **184**, 161–169 (2014)
25. Panti C., Giannetti M., Bainsi M., Rubegni F., Minutoli R. and Fossi M.C., Occurrence, relative abundance and spatial distribution of microplastics and zooplankton NW of Sardinia in the Pelagos Sanctuary Protected Area, Mediterranean Sea, *Environmental Chemistry*, **12**(5), 618–626 (2015)
26. Prata J.C., Microplastics in wastewater: State of the knowledge on sources, fate and solutions, *Marine Pollution Bulletin*, **129**(1), 262–265 (2018)
27. Rawat K. and Yadav A.K., Sustainable Practices for Conservation of Wetland Ecosystem, *Wetlands Conservation: Current Challenges and Future Strategies*, 129–139 (2021)
28. Rochman C.M., Hoh E., Kurobe T. and Teh S.J., Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress, *Scientific Reports*, **3**(1), 1–7 (2013)
29. Santos L.H., Insa S., Arxé M., Buttiglieri G., Rodríguez-Mozaz S. and Barceló D., Analysis of microplastics in the environment: Identification and quantification of trace levels of common types of plastic polymers using pyrolysis-GC/MS, *MethodsX*, **10**, 102143 (2023)
30. Sarkar A. and Jain S.K., Using Remote Sensing data to study wetland dynamics—A case study of Harike Wetland, In *Proceedings of Taal: The 12th World Lake Conference*, National Institute of Hydrology, Roorkee, India 680–684 (2007)
31. Savitha N. and Yamakanamardi S.M., Studies on abundance of zooplanktons in lakes of Mysore, India, *Journal of Environmental Biology*, **33**(6), 1079 (2012)
32. Sharma S. and Singh P., *Wetlands conservation: Current challenges and future strategies*, John Wiley & Sons (2021)
33. Singh S., Bhardwaj A. and Verma V., Remote sensing and GIS based analysis of temporal land use/land cover and water quality changes in Harike wetland ecosystem, Punjab, India, *Journal of Environmental Management*, **262**, 110355 (2021)
34. Su L., Xue Y., Li L., Yang D., Kollandhasamy P., Li D. and Shi H., Microplastics in taihu lake, China, *Environmental Pollution*, **216**, 711–719 (2016)
35. Vroom R.J., Koelmans A.A., Besseling E. and Halsband C., Aging of microplastics promotes their ingestion by marine zooplankton, *Environmental Pollution*, **231**, 987–996 (2017)
36. Wang W., Ndungu, A. W., Li, Z. and Wang, J., Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China, *Science of the Total Environment*, **575**, 1369–1374 (2017)
37. Wright S.L. and Kelly F.J., Plastic and human health: A micro issue?, *Environmental Science & Technology*, **51**(12), 6634–6647 (2017)
38. Yin L., Wen X., Huang D., Zhou Z., Xiao R., Du L., Su H., Wang K., Tian Q. and Tang Z., Abundance, characteristics and distribution of microplastics in the Xiangjiang river, China, *Gondwana Research*, **107**, 123–133 (2022)
39. Zhang L., Xie Y., Zhong S., Liu J., Qin Y. and Gao P., Microplastics in freshwater and wild fishes from Lijiang River in Guangxi, Southwest China, *Science of the Total Environment*, **755**, 142428 (2021).

(Received 10th January 2025, accepted 12th February 2025)