Non-Invasive Assessment of Microplastic Contamination in the Avian Biodiversity of Gharana Wetland, Jammu and Kashmir

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Abstract

Microplastic pollution has become a global environmental concern with potential impacts on ecosystems and wildlife. This study aimed to investigate the presence of microplastics in the avian fauna of the Gharana Wetland, employing a non-invasive monitoring approach through the analysis of bird droppings (scat). Gharana (meaning welcome home) wetland is a protected site under J&K Wildlife Protection Act, 1978. Gharana Wetland Conservation Reserve (GWCR) is recognized as "Important Bird Area" by Birdlife International, UK and Bombay Natural History Society (BNHS). It is an essential habitat for numerous avian species which are native as well as migratory one. Scat samples were collected of various avian species inhabiting the Gharana Wetland, representing diverse trophic levels within the ecosystem. Microplastic extraction and analysis were conducted using established protocols involving digestion, filtration and microscopic examination.

Preliminary results revealed the presence of microplastics scat of multiple avian species which was further confirmed by using FTIR technique for chemical analysis of particles. The types microplastics varied among the avian samples, suggesting diverse sources and routes of ingestion. Commonly identified microplastics included fragments, fibers and microbeads. The prevalence of microplastics in avian scat raises concerns about the potential ecological impacts on both individual birds and the broader wetland ecosystem. This work highlights the use of scat analysis as a non-invasive tool for monitoring microplastic pollution in bird populations, which is critical for conservation efforts. It emphasizes the importance of reducing microplastic contamination in freshwater environments and encourages more investigation into the long-term consequences on bird health and potential food chain transmission. Proactive efforts are required to mitigate environmental consequences.

Keywords: Microplastic, Scat, Avain fauna, Non-invasive, Consequences, Pollution.

Introduction

Plastics typically refer to a broad category of synthetic polymer-based materials that can be easily shaped using heat and have been extensively utilized in daily life since the 1950s[18]. Unfortunately, synthetic plastics do not biodegrade within a reasonable duration under natural conditions. Instead, they break down into smaller particles which are microplastics (MP; <5 mm) and nanoplastics (<0.1 μm), which persistently accumulate in the environment[44]. Once released into the environment, MP exhibit various physical and toxicological effects on wildlife[6]. Recent studies indicate that MP can hinder animal movement[29] and when ingested, they may cause damage and obstruction of the gastrointestinal (GI) tracts, leading to reduced food intake, starvation and direct mortality[9,40,46,55].

Toxic substances from MP, as well as hazardous contaminants absorbed on MP surfaces from the environment (e.g. DDT, PCBs, or heavy metals) have been shown to induce adverse health effects such as lowered survival and growth rates, delayed sexual maturity and reduced reproductive output[3,9,45,54]. This recognition has elevated MP pollution to one of the primary global conservation concerns[53]. While most research on MP has been conducted in aquatic environments[15], the contamination of terrestrial ecosystems is anticipated to be significantly more extensive[8,20,36]. Existing research indicates substantial MP soil contamination[12,26,43] with MP accumulating in plants[28] and various terrestrial invertebrates[26,34,47,51]. MP may then move up the food chain to herbivores, ingested with contaminated plant material, or to carnivores and scavengers that forage on contaminated prey[8].

MP has been found in tree Swallow Tachicyneta bicolor chicks, aerial insectivores[19,50] and in birds of prey such as Red-shouldered Hawks Buteo lineatus and Barred Owls Strix varia which hunt small and medium-sized vertebrates[5]. Nevertheless, studies on terrestrial habitats and wildlife remain mostly un-represented, emphasizing the need for large-scale monitoring programs to assess the extent of MP contamination in different ecosystems and trophic levels[8,42]. Birds, occupying various ecological niches in diverse terrestrial environments, have the potential to serve as indicators of MP contamination[14,16,17,32]. While research has shown the ingestion of MP by various wild terrestrial birds, from small songbirds to large raptors and scavengers, comprehensive population-level analyses are limited and existing data on MP ingestion by terrestrial birds often involve only a few individuals per species[5,19,57].

Therefore, there is still a significant knowledge gap regarding the extent and sources of MP contamination among wild terrestrial avian populations[1]. In this study, we explore the potential exposure of microplastics in the avain fauna of Gharana wetland. Many species are widely distributed in the wetland which are native as well as migratory species of birds[27,49]. Bar headed geese is the best to monitor for the contamination of microplastics contamination of Gharana wetland. The other species included for the surveillance of microplastics contamination in the birds are: purple moorhen, Mynahs and Egrets. The egrets and bar headed goose forage primarily on the ground[30]. Due to their widespread occurrence, these species provide an excellent opportunity to investigate and to compare MP pollution across the wetland. The primary objective of our study is to assess MP ingestion by bar headed goose and other species mentioned. Although all these species are generalists occupying different niches, their foraging strategies also differ slightly, potentially influencing MP ingestion.

Material and Methods

Study Area: Jammu and Kashmir has many designated wetlands on a national and international level. The National Wetlands Atlas (NWA) (2011) states that there were 3651 wetlands in the previously united State of Jammu and Kashmir, covering 3,91,501 hectares[7]. It is designated as a protected site under section 36(1) of the J&K Wildlife Protection Act, 1978. These protected area designations were initially adopted by the Wildlife (Protection) Amendment Act of 2002, which modified the J&K Wildlife Protection Act of 1978. The Gharana Wetland Conservation Reserve (GWCR) has been identified as a "Important Bird Area" by Birdlife International, UK and the Bombay Natural History Society (BNHS)[38]. The wetland is located 270 meters above mean sea level and has a surface area of 0.75 km^2 and is located in subtropical Jammu and Kashmir UT, northwest India, at a height of 270 meters above mean sea level. roughly 35 kilometres from the centre of Jammu City and 10 miles east of the international border between India and Pakistan.

The wetland is situated 270 meters above sea level at 32°32'28" N and 74°41'27" E. It is located in biogeographical zone 2A of the Northwest Himalayan. Gharana Wetland Conservational Reserve (GWCR), a marsh nourished by rain, is primarily preserved by nature. In addition, surface runoff from agricultural areas and spillover from the neighbouring Ranbir canal provide additional water supplies. Maximum summer temperatures in the wetland are 46°C and minimum winter temperatures can drop as low as 2°C. There is roughly 1331 mm of precipitation annually, most of which falls between July to September during the monsoon season[27].

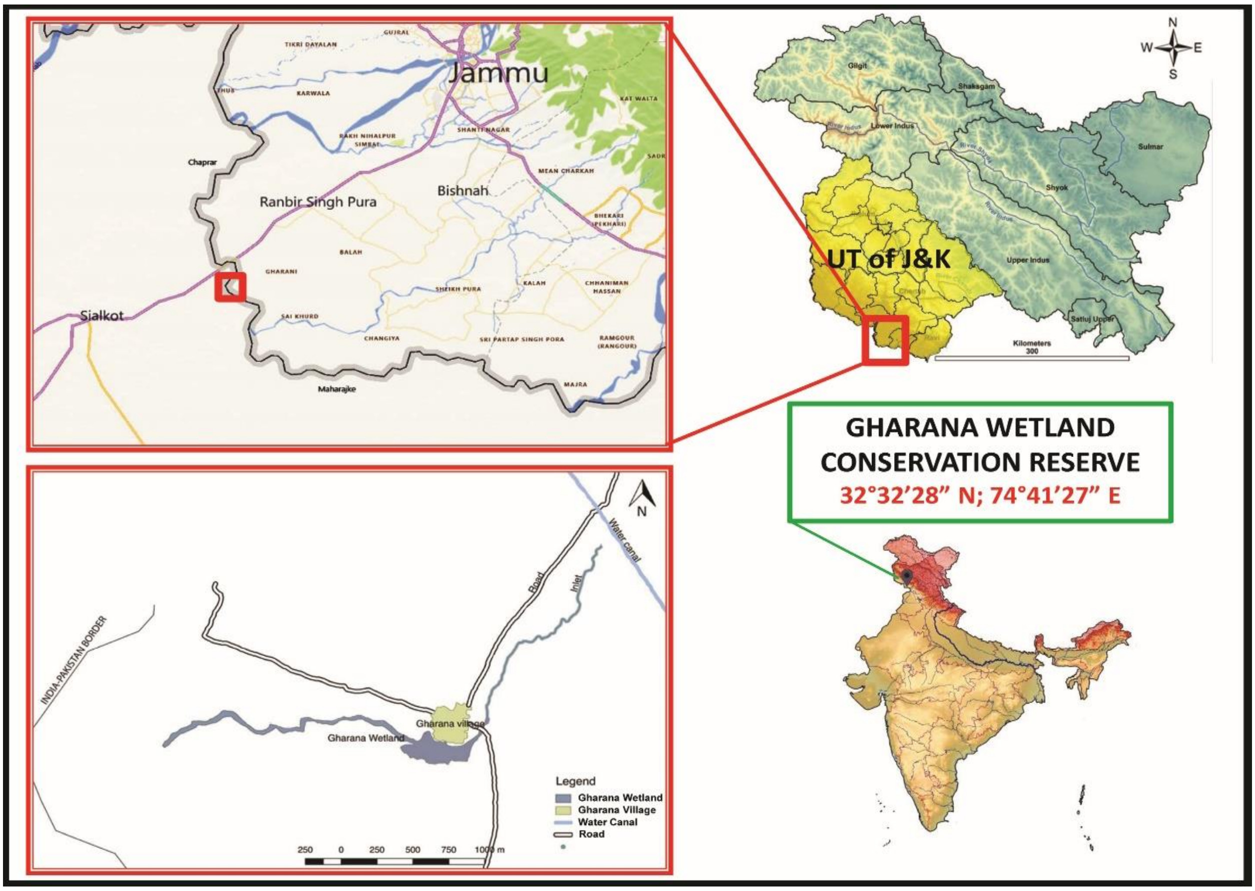


Fig. 1: GIS map of Gharana wetland in Jammu and Kashmir (UT)

Sampling: Feaces of birds were collected from monospecific flocks of bar headed goose, Egrets, Mynas and Moorhens in winter and post monsoon season 2022-23 whereas the feaces of Mynas and Egrets were collected in all the four seasons viz. winter, summer, moonsoon and post-moonsoon (2022-23) resting in particular sector of the wetland which were frequently approached by these different species[33].

Gharana Wetland is an important wintering site for the bar-headed Goose, providing a safe haven and plentiful food required for survival[30]. That is why the faeces of Goose were collected in winter and post monsoon season. Conservation activities in such locations are critical for protecting migratory patterns and the region's overall ecological health. After a waiting period of 30 to 45 minutes the flock was approached which had left the resting place[13]. Faeces were carefully collected from the ground with a spatula avoiding the sample ground particles[48]. The birds scat which were collected from the site by hand using nitrile gloves, were placed into sterile collection bags.

Sample Preparation and microplastic extraction: The samples were dried at room temperature, weighed and then frozen. Each scat was frozen (−20 °C) for further analyses in the laboratory. For analysis, each scat was defrosted and transferred to a clean glass beaker (250 ml) and 100 ml of potassium hydroxide solution (KOH, 10%) was added to digest the organic material. The samples then incubated for 24 h at 40 °C for digestion. The floating phase was vacuum filtered through a 1.2 µm glass microfibre filter.

Table 1
Details of sample collection across Gharana wetland

S.N.	Location	Season	Coordinates	Avain Fauna	No. of Scat	Mean and SD
1	Gharana Wetland	Winter	32°32'24.8"N 74°41'29.4"E	*Anser indicus* (Bar headed Goose)	30	31.6±6.2
			32°32'25.3"N 74°41'21.9"E		25	
		Post Moonsoon	32°32'25.5"N 74°41'31.6"E		40	
2	Gharana Wetland	Winter	32°32'27.7"N 74°41'35.9"E	*Ardea alba* (Great Egret)	10	5±3.08
		Summer	32°32'28.7"N 74°41'29.5"E		3	
		Moonsoon	32°32'29.1"N 74°41'26.3"E		2	
		Post Moonsoon	32°32'29.6"N 74°41'25.0"E		5	
3	Gharana Wetland	Winter	32°32'27.9"N 74°41'35.8"E	*Acridotheres tristis* (Common Mynas)	8	5.5±2.3
		Summer	32°32'28.8"N 74°41'33.4"E		7	
		Moonsoon	32°32'29.4"N 74°41'25.4"E		2	
		Post Moonsoon	32°32'30.0"N 74°41'36.0"E		5	
4	Gharana Wetland	Winter	32°32'26.8"N 74°41'35.1"E	*Porphyrio poliocephalus* (Purple Moorhen/ Swamphen)	20	28±8
		Post Moonsoon	32°32'28.8"N 74°41'33.2"E		36	

Table 2
Weight of freshly collected Scat/ Feaces of Avian fauna from Gharana wetland

Season	Bar headed Goose	Purple Moorhen/ Swamphen	Great Egret	Common Mynas
Winter	550g	300g	50g	30g
Summer	-	-	20g	30g
Monsoon	-	-	10g	5g
Post Monsoon	700g	400g	25g	15g

Table 3
Weight of collected suspected microplastic (residue) particles after following the procedure to separate microplastics from Scat/feaces of Avian fauna of Gharana wetland

Season	Bar headed Goose	Purple Moorhen	Egret	Mynas
Winter	350g	150g	75g	15g
Summer	-	-	40g	15g
Monsoon	-	-	50g	8g
Post Monsoon	328g	180g	100g	20g

The residue is transferred to a new glass beaker and hydrogen peroxide (H_2O_2, 10%, 24 h) was added to increase the recovery of potential particles trapped in the residue and the filtration was repeated. Each filter was placed in a Petri dish and dried in an oven at 40 °C. Then disaggregation of each faeces sample in water was done using tweezers and a mounted needle and these can be analyzed by mean a binocular magnifying glass. Particles were subjected to visual inspection followed by FT-IR technique for identification and chemical analysis of microplastics in scat of aves[11,13].

Quality Control Precautions: While conducting non-invasive microplastic analysis in avian scat, scat samples were collected using clean, non-plastic tools such as gloves and sterilized glass containers to prevent contamination[37]. Collected samples were stored in clean, airtight containers during transport and kept in a controlled lab environment[35,39]. To guarantee the objective assessment of anthropogenic contaminants, we followed stringent quality assurance and quality control procedures during the whole sampling and analysis process[25].

Throughout the entire process, precautions were taken to avoid microplastic and synthetic fibre contamination. With the exception of nitrile gloves which were needed for experimental procedures, plastic materials were avoided throughout the whole processing process.

In the laboratory, all personnel used 100% white cotton laboratory coats[4]. Prior to use, all tweezers were rinsed with pure water and glass beakers were covered with a double layer of aluminum and heated in an oven at 450 °C for 4 h to ensure the removal of all potential contaminants[41].

Observation, Identification and Analysis of Microplastics:
Visual Analysis: The residual particles as shown in table 3 were subjected to visual inspection under seteromicrocope followed by hot needle test, then microphotography was done with the help of Leica phase contrast light microscope at the resolution of 50 µm.

Chemical Analysis: Fourier transform infrared (FTIR) spectroscopy scanned samples and analysed their chemical characteristics using infrared light. FTIR identifies functional groups in chemicals in a sample. For FTIR spectroscopy in microplastic or polymer analysis, samples were scanned across a wavenumber range of 4000–400 cm^{-1} with a resolution around 4 cm^{-1}, balancing detail and sensitivity.

To improve signal quality, 16–64 scans per sample were often performed, while dry nitrogen gas was used to purge the atmosphere, reducing interference from CO_2 and H_2O[56]. Samples were usually dried beforehand and were analyzed directly using an ATR accessory[2]. Consistent room temperature conditions and a preliminary background scan were also done to ensure reliable, high-quality spectra[52].

Statistical Analysis: An exploratory analysis showed a non-normal distribution of microplastic concentration (Shapiro-Wilk normality test; $p < 0.05$).

Results and Discussion

Validation and composition of microplastics: In this research study, microplastics were identified in the droppings (scat) of various bird species in the Gharana Wetland Conservation Reserve. FTIR Analysis revealed that approximately 70% of the residue consisted of microplastics. The predominant polymers present were polyethylene and polypropylene followed by other identified polymers including nylon, polycarbonate, polystyrene, ethylene vinyl acetate, acrylonitrile butadiene and latex. Table 4 details the specific types of polymers detected in the scat of each bird species. Additionally, non-plastic materials such as cellulose and cotton were found in the fecal samples of the avian fauna inhabiting the wetland.

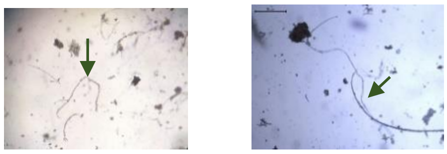
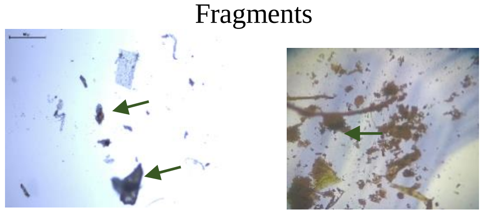
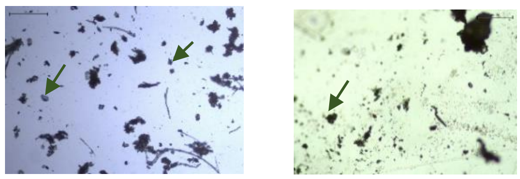
Abundance of microplastics: Fibers were the most commonly identified type of microplastic across the samples, with fragments and films being less prevalent. Polypropylene (PP) and polyethylene (PE) primarily appeared as fibers and fragments, contributing significantly to the total microplastic content. Other polymers like latex and ethylene vinyl acetate (EVA) were mainly present in film or foam forms, while polycarbonate (PC) and Polystyrene (PS) were predominantly found as rigid fragments. These findings indicate that fibers, particularly in blue, white and red colors, were more abundant than other forms, suggesting that they play a significant role in microplastic pollution in the surveyed area. Moreover, microplastics, specifically fibers and fragments, were found in all analyzed scat samples, with the detection of different polymers varying seasonally across the surveyed areas.

Fibers were the predominant type of microplastic identified in the surveyed samples. They appeared more frequently than fragments and were consistently found across different species and sampling locations. In the analysis of microplastics found in avian fauna, several trends emerged based on the species and the seasons sampled. In bar-headed Goose samples, 64% of the winter samples contained fibers (PP and PE) while the post-monsoon season showed a mixture of polymers including PP, PE, polycarbonate, latex and acrylonitrile butadiene. For the purple Moorhen, 66% of winter samples had fibers (PP and PE), while post-monsoon samples included PP, PE, PS, nylon and ethylene vinyl acetate.

Table 4
Details of types of Microplastics identified in different avain fauna

S.N.	Avain Fauna	Season	Polymers identified	Maximum Microplastic in sample
1.	Bar Headed Goose	Winter	PP, PE, Nylon, Polycarbonate	PP and PE
		Post Moonsoon	PP, PE, Polycarbonate, Latex, Acrylnitrile Butadiene	
2.	Purple Moorhen	Winter	PP, PE, PS, Nylon	PP and PE
		Post Moonsoon	PP, PE, PS, Nylon, Ethylene Vinyl Acetate	
3.	Great Egret	Winter	PP, PE, PS, Nylon	PP and PE
		Summer	PP, PE, PS, Nylon, Ethylene Vinyl Acetate	
		Moonsoon	PP, PE	
		Post- Moonsoon	PP, PE, Latex, Nylon, Ethylene Vinyl Acetate	
4.	Common Mynah	Winter	Polycarbonate, PS, Acrylnitrile Butadiene	PS
		Summer	PS, Ethylene Vinyl Acetate, Latex	
		Moonsoon	Nylon, PS	
		Post- Moonsoon	Nylon, ABS, PS	

Table 5
Types of Microplastics found in Scat samples of Avain Fauna of Gharana wetland Conservational reserve of Jammu and Kashmir

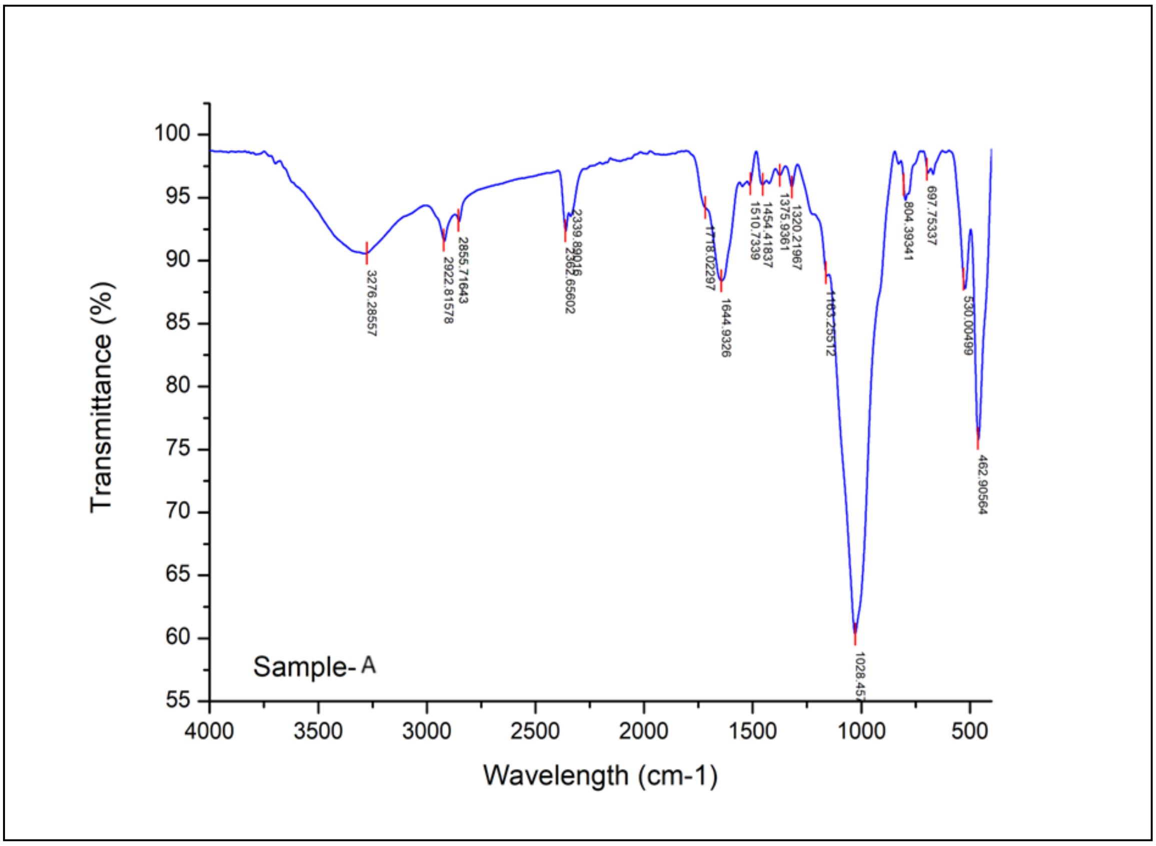
S.N.	Type of Microplastic	Microphotograph
1.	Fibers	Colored Fibres 
2.	Fragments	Fragments 
3.	Films	Films/ Foams 

In Great Egret samples, fibers were found in 63% of winter samples (PP and PE), while summer samples included PP, PE, PS, nylon and ethylene vinyl acetate. Monsoon samples showed the presence of PP and PE and post-monsoon samples contained PP, PE, latex, nylon and ethylene vinyl acetate. Common Mynah samples indicated that in winter, Polycarbonate, PS and acrylonitrile butadiene were found, with PS being the maximum microplastic. In summer, PS, ethylene vinyl acetate and latex were detected, while monsoon samples contained nylon and PS and post-monsoon samples revealed nylon, ABS and PS. Overall, fibers were the most prevalent type of microplastic across the species studied, particularly in blue, white and red colors.

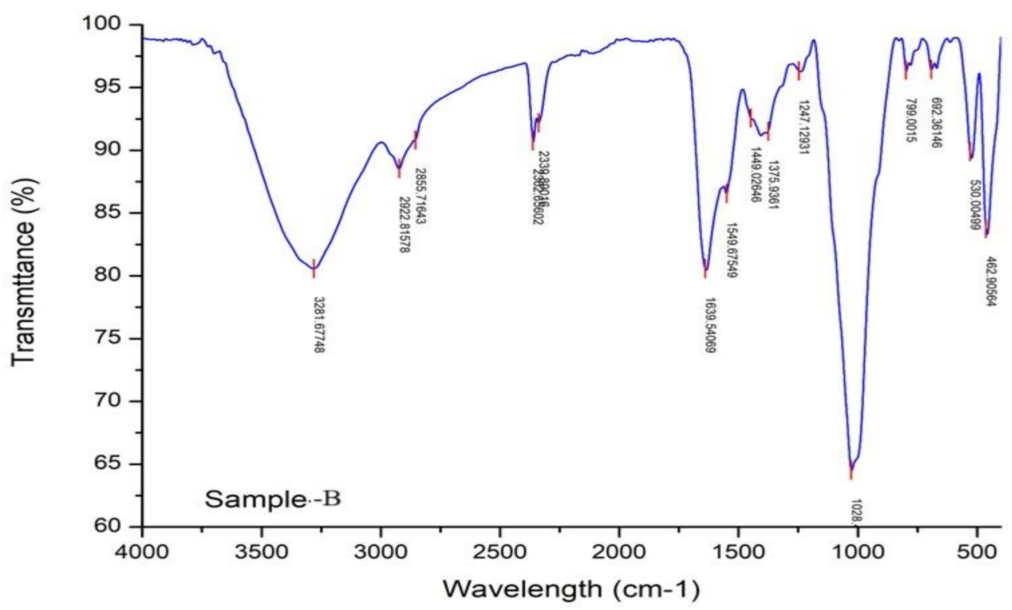
These findings underscore the importance of monitoring microplastic pollution, as fibers appear to be the most abundant type in the environment. Micro-FTIR analysis confirmed that these microplastics were of anthropogenic origin. Additionally, natural polymers like cotton were detected alongside other synthetic polymers such as PET and nylon.

Types of microplastics: The identified polymers were identified as fibres, fragments and films by taking microphotograph at 50 µm of the Leica phase contrast microscope further confirmed by using FTIR.

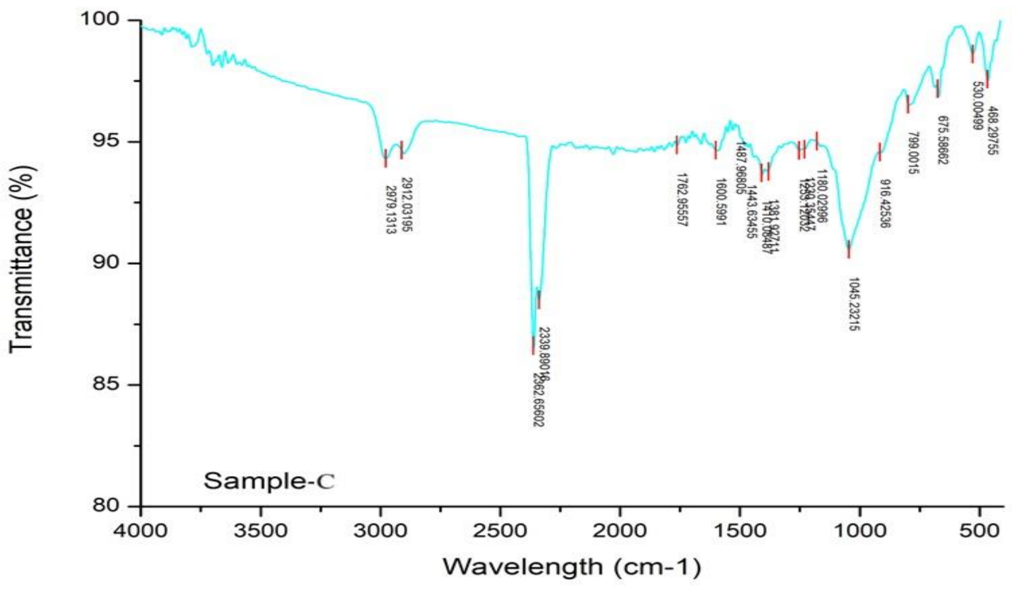
The polymer diversities found in the scat samples of the avaian fauna using Fourier transform infrared spectroscopy (FTIR) analysis are: polyethylene (PE), polyproplyene (PP), polystyrene (PS), nylon, ethylene vinyl acetate, latex, polycarbonate and acrylonitrile butadiene



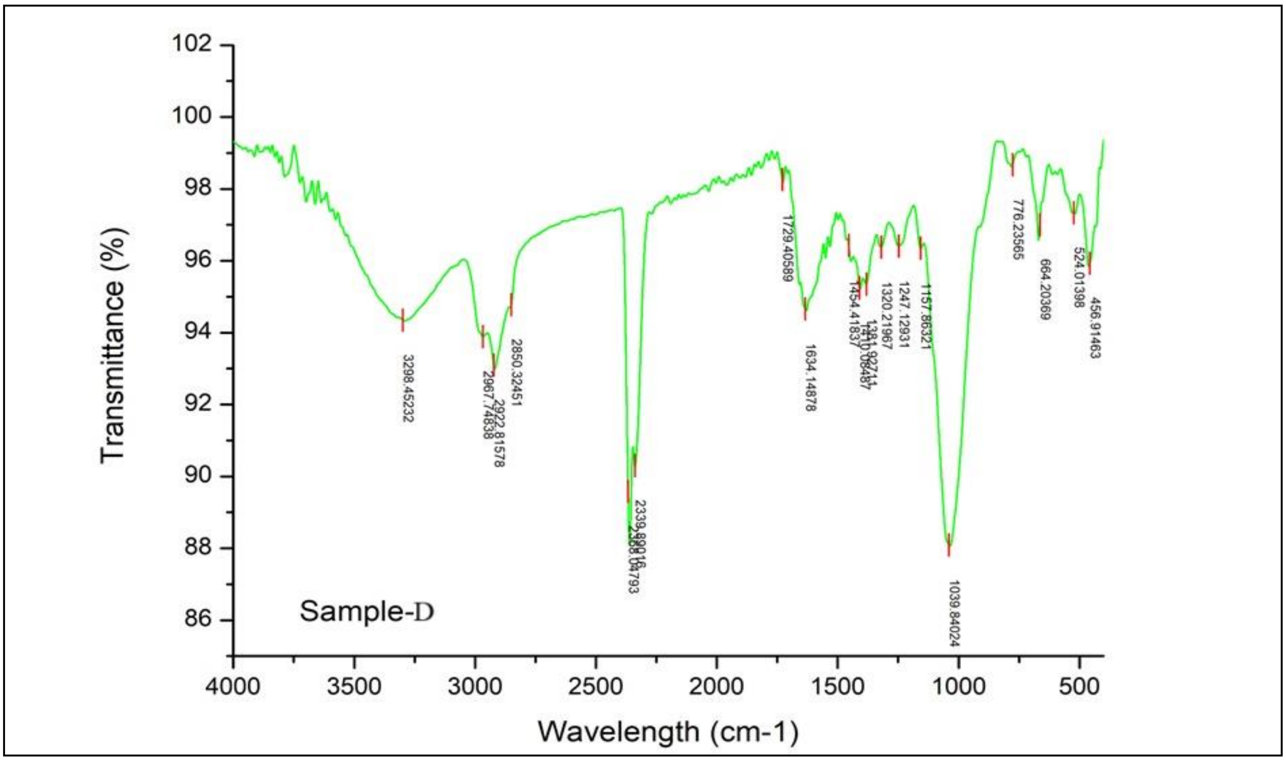
Characteristic peaks (cm^{-1}) (IR Wavelength)	Functional Group Assignment	Microplastic Polymer
2922	C-H stretching (PE)	**Polyethylene (PE) Polypropylene (PP) Polystyrene (PS)**
2855	C-H stretching (PE, Nylon and PS)	
1453	CH_2 bending (PS and PP)	
1465	CH_2 bending (HDPE and LDPE)	
1375	CH_2 bending (PP and PE)	
1028	Aromatic CH Bending (PS)	
804	CH_3 rocking, C-C stretching CH_2 rocking, C-CH_3 stretching CH_2 rocking, C-C stretching, C-CH stretching (PP)	
522	Aromatic ring out-of- plane bending (PS)	



Characteristic peaks (cm^{-1}) (IR Wavelength)	Functional Group Assignment	Microplastic Polymer
3281	N-H stretching (Nylon)	Nylon
2922	C-H stretching (Nylon)	
2855	C-H stretching (Nylon and PS)	
1639	C=O stretching (Nylon)	
1549	NH bending, C-N stretching (Nylon)	
1449	CH_2 bending (PS, PP, Nylon)	
1375	CH_2 bending (PP, Nylon)	
1028	Aromatic CH Bending (PS)	
692	Aromatic NH bending, C=O bending (Nylon)	



Characteristic peaks (cm^{-1}) (IR Wavelength)	Functional Group Assignment	Microplastic Polymer
2979	C-H stretching (PC)	**Polycarbonate (PC)** **Acrylonitrile butadiene (ABS)**
2912	C-H stretching (ABS)	
1762	C=O stretching (PC)	
1600	Aromatic Ring stretching (ABS)	
1487	Aromatic Ring stretching (ABS)	
1443	CH_2 bending (ABS)	
1410	Aromatic Ring stretching (ABS)	
1381	CH_3 bending (PC)	
1180	C-O stretching (PC)	
1045	Aromatic CH in plane bending (PC)	
675	Aromatic CH out-of-plane bending (ABS)	



Characteristic peaks (cm^{-1}) (IR Wavelength)	Functional Group Assignment	Microplastic Polymer
3298	N-H stretching (Nylon)	Nylon Polypropylene (PP) Latex Ethylene Vinyl Acetate (EVA)
2967	C-H stretching (PP, Latex)	
2922	CH stretching (PP, Nylon, EVA, Latex)	
2850	CH stretching (PE, PP, Nylon, EVA, Latex)	
1729	C=O stretching (EVA)	
1634	C=O stretching (Nylon, EVA)	
1454	CH2 bending (PP, Nylon, Latex)	
1381	CH3 bending (Latex)	
1247	C(=O)O stretching (EVA)	
1157	CH bending, CH3 rocking (PP) C=C stretching (Latex)	
1039	C-O stretching (EVA)	
664	NH bending, C=O bending (Nylon)	

Fig. 2: FTIR spectrum transmittance along with the tables depicting FTIR peaks for identification of particular polymers present within the Scat samples of Avaina fauna of Gharana Wetland: (A) PE, PP, PS (B) Nylon (C) ABS, Polycarbonate (D) Nylon, PP, Latex and EVA

Conclusion

This study thoroughly examined the presence of microplastics in the droppings of various avian species within the Gharana Wetland conservation reserve. The findings underscore the significant prevalence of microplastics, particularly fibers, highlighting a critical environmental concern that warrants further investigation and action. Across the surveyed species: Bar-headed Goose, Purple Moorhen, Great Egret and Common Mynah, fibers emerged as the most abundant microplastic type, with detection rates ranging from 63% to 71%. Specifically, in Bar-headed Goose samples, 64% contained fibers, while 14% exhibited fragments.

Similarly, Purple Moorhen samples revealed that 66% contained fibers and Great Egret samples indicated 63% fibers and 8% fragments. Common Mynah samples demonstrated a notable prevalence of fibers, found in 71% of the samples. These results suggest that fibers, particularly those made from polyethylene (PE) and polypropylene (PP), are more frequently ingested compared to other microplastic forms such as fragments or films. The implications of these findings are substantial. The high occurrence of microplastics in avian droppings indicates that these birds may be ingesting microplastics through their diet or from contaminated habitats, which could have detrimental effects on their health and the broader ecosystem.

The anthropogenic origin of these microplastics, as confirmed by Micro-FTIR analysis, highlights the urgent need for improved waste management practices and pollution mitigation strategies to protect these vulnerable habitats. Additionally, the study identified natural polymers, such as cotton, coexisting with synthetic microplastics, suggesting a complex interplay between environmental factors and human activities. This combination raises concerns about the potential for microplastics to disrupt food webs and ecosystem dynamics, as these materials can be mistaken for food by wildlife. Given the alarming levels of microplastic contamination found in this study, it is crucial for future research to investigate the long-term ecological impacts on avian health, population dynamics and habitat integrity.

Monitoring efforts should be expanded to include other wildlife and environmental compartments such as water and soil, to gain a comprehensive understanding of microplastic pollution in wetland ecosystems. This study deals with microplastic contamination in the droppings of Avian species within the Gharana Wetland conservation reserve, establishing a crucial foundation for future research and conservation initiatives. In conclusion, this research serves as a foundation step in addressing the growing issue of microplastic pollution in Avian habitats. The findings call for urgent conservation efforts, public awareness campaigns and policy changes aimed at reducing plastic pollution and safeguarding Avian biodiversity in the Gharana Wetland conservation reserve and similar ecosystems.

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