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Research Article

Microplastic in the commercially important gastropod, *Babylonia spirata*, from Indus Delta, Pakistan

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Abstract

Mollusks, particularly gastropods, are crucial for ecosystem functions such as nutrient cycling, food web dynamics, habitat engineering, bioindication, erosion control, and species interactions. They function as carriers of contaminants for higher trophic-level organisms within the food chain. They are also thought to be sensitive markers of changes in the environment, particularly contaminants like heavy metals and microplastics. Due to the high prevalence of microplastics in both freshwater and marine organisms, microplastics are receiving more attention globally. Still, there is a dearth of knowledge regarding animals found in estuaries. In this baseline investigation, the distribution and abundance of microplastics in *Babylonia spirata* from Indus Delta, Sindh Pakistan are assessed.The mean abundance of microplastics in *B. spirata* was 28.81±12.94 items/ind and 16.54±12.53 items/g of tissue. Fibers were the dominant type of microplastics (69%), among which black color (37%) was common. PE (25.8%) was the dominant polymer type of microplastic which was identified by FTIR. Evaluating the degree to which *B. spirata* were contaminated by microplastics sheds light on the potential use of gastropods as bioindicators for baseline research and monitoring.

Introduction

International authorities, policymakers, scientists, stakeholders, and the general public have all expressed great concern about the marine pollution produced by microplastics, or particles less than five millimeters in diameter (Fang *et al.,* 2019). Microplastics can have an adverse chemical or physical effect on marine life. These particles can block the biota's digestive systems and act as carriers of metals and organic contaminants, which can absorb, concentrate, and build up in the tissues of living things. Because of this accumulation, eating these creatures can make marine life poisonous (Li *et al.,* 2021). The effects of microplastic on fish, bivalves, and crustaceans have been the main focus of previous toxicity investigations (de Sá *et al.*, 2018). On the other hand, effects on gastropods are not well explored and are hence underrepresented in risk assessments that are now in use. Both in the field and in the lab, microplastic consumption by gastropods has previously been shown (Gutow *et al.,* 2016, 2019). Eighty percent of the molluscan phylum is made up of the diverse group of animals known as gastropods (Marshall *et al*., 2020). Both humans and other marine species rely on marine gastropods as a major source of food (Boyd *et al*., 2020).

Seagulls and crabs, among other larger marine species, frequently consume these gastropods as prey. Gastropods can be divided into five major lineages (Cunha and Giribet, 2019). The feasibility of tracking human pollution in coastal waters through the use of gastropods as a biological indicator has been the subject of increasing amounts of researchin the past few years. (Thushari *et al*., 2017; Aranda *et al*., 2022). Firstly, gastropods are a perfect organism for monitoring marine microplastic in both intertidal and marine sediments because of their worldwide distribution. Secondly, a variety of environmental stressors, including anoxia, freezing temperatures (Storey *et al.,* 2013), salinity (Ho *et al*., 2019), and desiccation (Schweizer *et al.,* 2019), are known to be tolerated by gastropods. Thirdly, because they are feeders of detritus, gastropods suck up food that is suspended in the water column and feed directly on sediments. Filter, ciliary, and mucus feeding are three strategies for capturing suspended particles that the organism may employ singly or in combination. Because of this, in aquatic situations, gastropods can be used as an effective bioindicator of ambient microplastic concentration. Gastropods, which are common forms of seafood in many nations, such as conches, periwinkles, and abalones, should also be studied (Curren *et al.,* 2024).

In order to determine if microplastics are present in the body, the current study evaluates microplastic contamination in *Babylonia spirata* and looks at the shape, size, color, and polymer type of microplastics. *B. spirata* had a higher number of microplastic items, which could be attributed to its larger size, greater retention capacity, and detritivores feeding habit (Abisha *et al.,* 2024).

Kuroda *et al.* (1971) identified the gastropod *B. spirata* (Linnaeus, 1758) as a member of the Family Babyloniidae of the Order Neogastropoda. This species, which goes by the names spiral whelk, babylon, "baigai," and ivory shell, is significant to the economy. While Moazzam and Ahmed (1994) reported on the species' fishing activities along Pakistan's coast, Kazmi *et al*. (2018, 2022) presented details of the species' occurrence in Pakistan. When frozen ivory shells were shipped to Taiwan in 1992, commercial ivory shell fishing got underway. Since then, the gathering of it has grown to become a significant fishing industry along Pakistan's coast. Today, targeted trap fisheries are conducted along Pakistan's coast, and the gathered shells are shipped to nations in the Persian Gulf and Southeast Asia both frozen and alive (Moazzam and Moazzam, 2023).

Materials and methods

Study area

The mudflats of Katiyar Jo Tar (24°38'42.204" N, 67°30'23.664" E), along the Wari Khuddi Creek near Darya Peerabad, were the source of the *B. spirata*, which are thought to be the main fishing grounds for them. The samples were collected from seven locations along Wari Khuddi Creek (Fig.1). The distance between each station was roughly 1.9 kilometers. The samples were gathered from January to December 2023. In the lower littoral zone, the density of *B. spirata* was found to be roughly 231/m^2 throughout the year (Moazzam and Moazzam, 2023).

Figure 1: Satellite Imagery with Latitude and Longitude.

Sample collection

There are seven stations, each 1.9 km apart, along the Wari Khuddi Creek. The natural beds of edible *B. spirata* at this location were chosen for sampling. Approximately 331 specimens were taken from seven stations (Table 1). About 44-49 specimens were randomly selected from each site and placed into glass jars. Local fishermen who used the tarp method and hand collection from the mudflats helped gather all the samples from the site. After being collected, all of the samples were brought fresh to the lab for further analysis after their storage in an icebox while covered in aluminum foil. The samples were sent to the lab for further analysis aseptically, both in aluminum foil packets and glass bottles, at 4°C (Dutta *et al.,* 2022).

Table 1: Morphometric properties of *Babylonia spirata*, a commercially significant species, taken from the Indus Delta, Pakistan's Wari Khuddi Creek

Station	Number of Species collected	Shell length on average (mm)	Shell height on average (mm)	Shell width on average (mm)	The total weight (g)	Weight of soft tissue (g)	Abundance of Microplastic (%)
Station1	49	34.99±3.38	30.53±3.09	24.16±2.32	15.5±4.31	1.93±0.52	100
Station2	48	34.65±2.48	30.11±3.01	24.08±2.13	15.37±4.22	1.77±0.46	100
Station3	44	32.93±2.72	29.47±2.77	23.58±2.01	15.09±4.27	1.74±0.47	100
Station4	49	34.87±3.32	30.33±3.00	23.76±2.08	15.36±4.19	1.75±0.41	100
Station5	48	33.86±2.92	29.99±3.56	24.73±2.59	16.73±4.81	1.82±0.62	100
Station6	47	32.71±2.87	29.5± 2.86	23.73±2.38	15.45±4.38	1.83±0.59	100
Station7	46	33.79±3.28	30.33±3.31	24.58±2.87	15.63±4.48	1.87±0.42	100

Mean±standard error values

Isolation of microplastic

Following the complete removal of the body from the shells, distilled water was used to rinse away any remaining sediments and other objects. The shell's height, breadth, and length were measured, among other morphometric parameters.The soft tissues of each specimen were preweighed and put into a conical flask, which was then filled with 40 mL of 10% KOH and allowed to sit at 40°C for 48 hours (Karami *et al.,* 2017; Thiele *et al*., 2019). Using 100 mL of a 33.7% (337 g in 1 L) hypersaline NaCl solution (1.2 g cm−3), density separation was carried out overnight. In order to facilitate filtering, the 10% 1 N (36.5 g) HCl solution was used to neutralize the alkaline pH of the digested solution to pH 7.Using vacuum filtration equipment, the sample solutions were quickly filtered through 1.6 µm pore filter paper (WhatmanTM Glass Microfiber Filters GF/C; 47 mm/1.6 µm). After that, the filter papers were carefully placed on Petri dishes and dried for four to eight hours at 40°C in a hot air oven.

Microplastics analysis

The dried filter papers were examined using an Olympus SZ61 stereomicroscope, which has a zoom range of 24 to 386 times and images were taken by using Axiocam Digital Camera. Visual inspection was used to identify and classify the microplastic particles into various groups according to their size (Abbasi *et al.,* 2018; Gurjar *et al.,* 2021a), morphotype (Li *et al.,* 2015, 2016), and color (Li *et al.*, 2018). ImageJ software was used to measure the particle size of microplastic samples. Many common and unknown particles were chosen, and their identities were confirmed using Fourier Transformed Infrared Spectroscopy (FT-IR) (Li *et al.,* 2019).

Application of Fourier Transform Infrared (FTIR) spectroscopy for the identification of microplastic polymers

Fourier Transform Infrared (FTIR) spectroscopy (Bruker Alpha II, Germany) was used to analyze the microplastic and different microplastic polymers in *B. spirata*. For the examination of carbon nanotubes and polyethylene, bands in the 500–4,000 cm^{-1} range were chosen. The height of samples containing carbon nanotube absorbance peaks was compared to the control to determine the results.

Contamination control and safety

To prevent contamination, every extraction procedure was performed in a sterile setting. To remove particulates, 1.6 µm pore size filter paper was utilized for pre-filtering chemical solutions, including distilled water, KOH, HCl, and NaCl. To account for any procedural contamination, a single blank extraction group devoid of tissue was carried out concurrently. Glass measuring cylinders, beakers, conical flasks, and bottles were utilized to reduce the amount of plastic that was exposed. Before the soft tissues were extracted, distilled water was used to rinse each molluscan sample. Distilled water was used to clean all of the tools, utensils, and stereomicroscope. The workspace was also thoroughly cleaned every day. Latex gloves and cotton lab coats were required for the analysis. Two glass bottles filled with filtered distilled water were used as a field blank to measure any potential contamination in each sampling location throughout the field sampling process (Gurjar *et al.*, 2023). To evaluate the airborne and procedural contamination of microplastics during the sample collection, removal, and identification process, two laboratory blanks of distilled water and blank filter paper were also used (Nuelle *et al.*, 2014; Gurjar *et al.*, 2021b).

Statistical data analysis

The importance of microplastics abundance was inspected by means of a one-way analysis of variance (ANOVA) set at the 0.05 level of significance. The normality of the data, the significant difference between the two samples, the correlation between the abundance of microplastics and the morphometrics measured with a significance level of $p<0.05$, the significance of the difference between the abundance of microplastics of different sizes, and the dispersal of microplastics in *B. spirata* with respect to its environment were all determined using the Shapiro-Wilk test, Independent Samples t-test, Pearson correlation matrix, Mann-Whitney U test, and regression correlation investigation, in that order. IBM SPSS 20 was used to perform all statistical analyses.

Results

The *B. spirata* is a scavenger's feeder on the bottom. The study's findings demonstrated morphometric information of shells and contamination of microplastics in every specimen of seven distinct sites located along the Wari Khuddi Creek, Indus delta (Table 1). In soft tissue, the mean microplastic abundance in *B. spirata* had the highest mean abundance (30.87±11.47 items/ind).

Based on their morphotypes, sizes, and colors, the microplastics found in *B. spirata* were classified into several types (Fig. 2). Out of all the specimens gathered, fiber (68.73%) was the most common morphotype reported. Fragments (22.31%), films (6.52%), and microbeads (2.44%) were the next most common morphotypes. Microbeads contributed considerably less than other categories. The microplastic size range was found to be greater than 7 μm. Simultaneously, the majority of the microplastics in the samples belonged to the size group of >1000 μm (38.65%), followed by 510–1000 μm (27.48%). The majority of the recovered microplastics in the 500–1000 μm and >1000 size groups were fibers, and the microplastics in the −100 μm size group were fragmented (Fig. 2a). The dominant colors were black (35.63%), followed by transparent (26.31%), red (19.85%), and blue (15.19 %) (Fig. 2b).

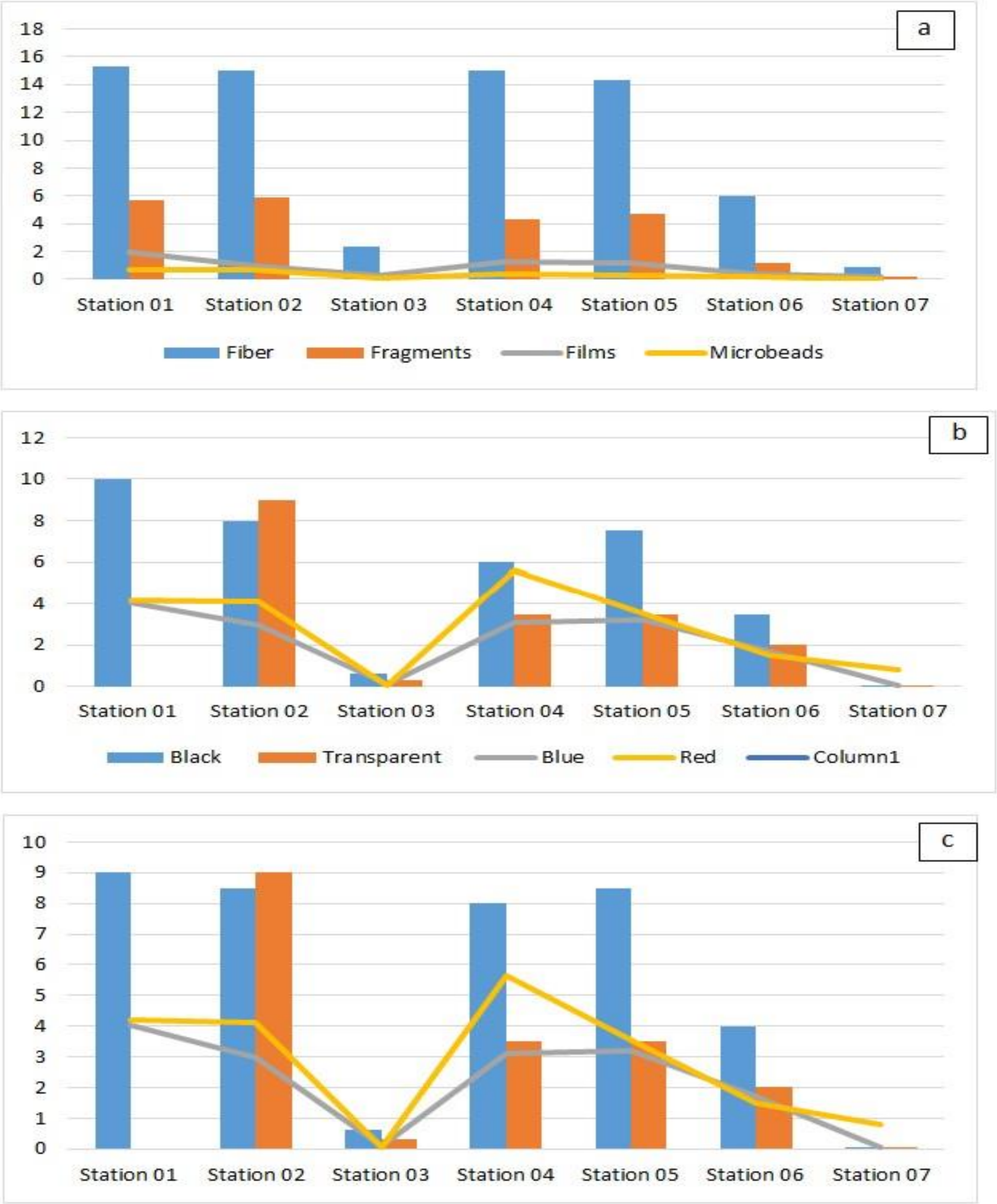

Figure 2: Comparative study of the different types of microplastics (percentage wise) based on size (a), morphotype (b), and color (c) in the gastropod samples from the seven different sites.

The analysis of variance (ANOVA) results indicates that there was a noteworthy distinction (F=13.173, p=0.001) in the mean abundance of microplastic, samples taken from the seven locations. Simultaneously, there were no discernible changes ($p>0.05$) across the samples from various stations. Stations 1,2,4 and 5 had the highest concentrations of microplastic in the samples, respectively.

The Shapiro-Wilk test revealed a difference ($p<0.05$) in the amount of microplastics among the stations. The quantity of microplastic particles/gram of soft tissue was (H=113.7, $p<0.05$).

The abundance of microplastic (items/individual) in *B. spirata* showed a linear significant connection. Other than this, there was no discernible relationship between the environmental matrix and microplastic abundance in *B. spirata*. The morphotype, size, and color of microplastic are compared between the specimens of different stations (Fig. 3).

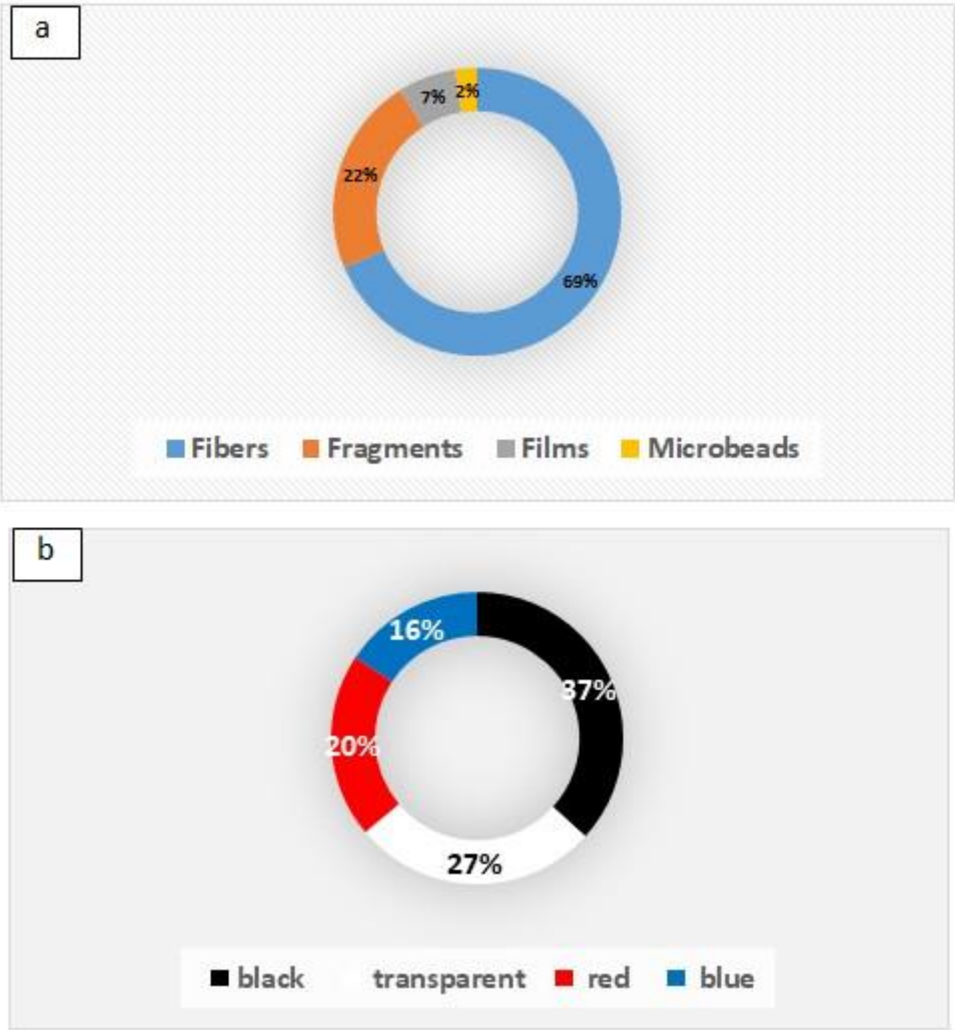

Figure 3: The percentage distribution of various MP types that were recovered from the samples of the seven distinct sites, categorized by morphotype (a), and color (b).

The results of the Independent Samples t-test indicated that *B. spirata* were found to have a high concentration of microplastics with transparent colors, and fibers measuring more than 1000 µm were the most common category. The Mann-Whitney pairwise test results showed that there was a substantial (p<0.05) variation in the frequency of fibers (U=2624.3, p<0.001) and fragments (U=3179.4, p<0.05).

Perhaps as a result of their source and the variations in their environments, the mean microplastic abundance recorded in the *B. spirata* differed considerably. However, there was no distinction noted in the size and shape of microplastics in the *B. spirata*. There was a noticeable difference in the color of microplastics among *B. spirata* respectively, which had a greater predominance of black and transparent colors, respectively.

Interestingly, the number of Microplastics/ind from the seven distinct stations varied significantly (p>0.05) even though the average microplastic abundance of the *B. spirata* samples taken from these stations was reported as follows: 28.81±12.94 items/ind and 16.33±10.43 items/g of tissue (at station 1); 28.62±12.76 items/ind and 16.54±12.53 items/g of tissue (at station 2); 27.35±11.71 items/ind and 16.01±10.54 items/g of tissue (at station 4); 27.46±11.58 items/ind and 16.22±10.47 items/g of tissue (at station 5); 24.35±10.71 items/ind and 16.01±10.54 items/g of tissue (at station 6); 21.62±9.94 items/ind and 14.86±10.22 items/g of tissue (at station 3); 21.46±9.57 items/ind and 14.77±10.15 items/g of tissue (at station 7);. Concurrently, there existed a noteworthy variation (p<0.05) in the microplastics/g of tissue among the sites (F=16.752, p<0.001). All other types of microplastics were fairly equivalent throughout the seven stations, despite the fact that there were significant differences (p<0.05) in the occurrence of fibers (F=6.842, p=0.023) among *B. spirata* samples from the seven locations. Four different types of polymers were identified by FTIR analysis of the sediments:

Polyethylene (PE), polyamides (Nylon), polystyrene (PS), and polyvinyl chloride (PVC) (Fig 4).

The sample spectra are displayed in Figure 5. The figure showed the same absorption, with the only variable being in strength, which may be attributed to variations in the film compound. The FTIR spectrum of nanocomposite including nanotubes of carbon is displayed in Figure 5. The=C–H (flexural) at 100-500 cm-^{1}, C=C (vibratory) at 2,550–2,850 cm-^{1}, and C–H (vibratory) at 3,100-3650 cm-^{1} were detected, according to FTIR analysis which shows the different polymer compounds of PE, Nylon, PS, and PVC.

The overall length, shell weight (body weight), and tissue weight of *B. spirata*that consumed MP exhibited a positive correlation with *B. spirata*(r= −0.3558, r= −0.4359, and r= −0.5397), respectively. This suggested that the size of the *B. spirata*had an impact on the amount of MPs consumed: the larger and heavier the *B. spirata*, the more MPs were ingested.

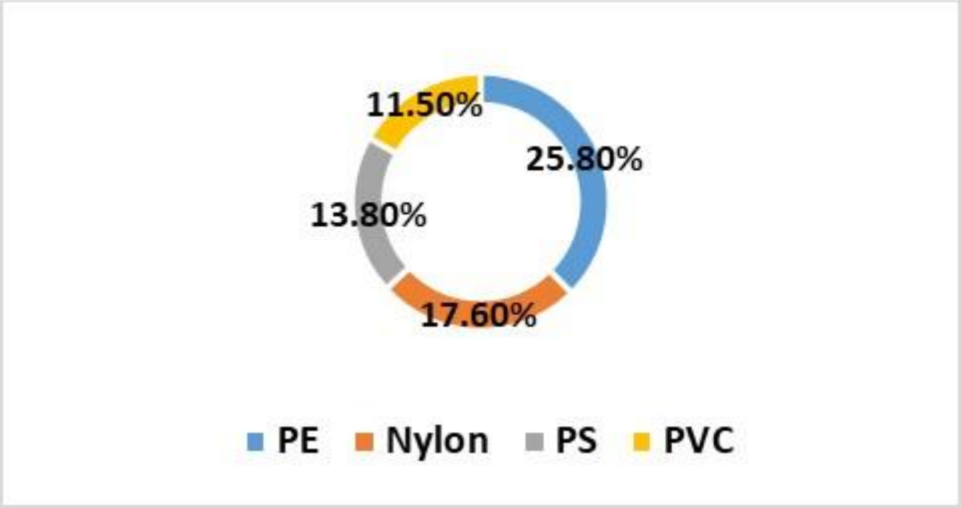

Figure 4: The percentage distribution of polymer types in *Babylonia spirata*.

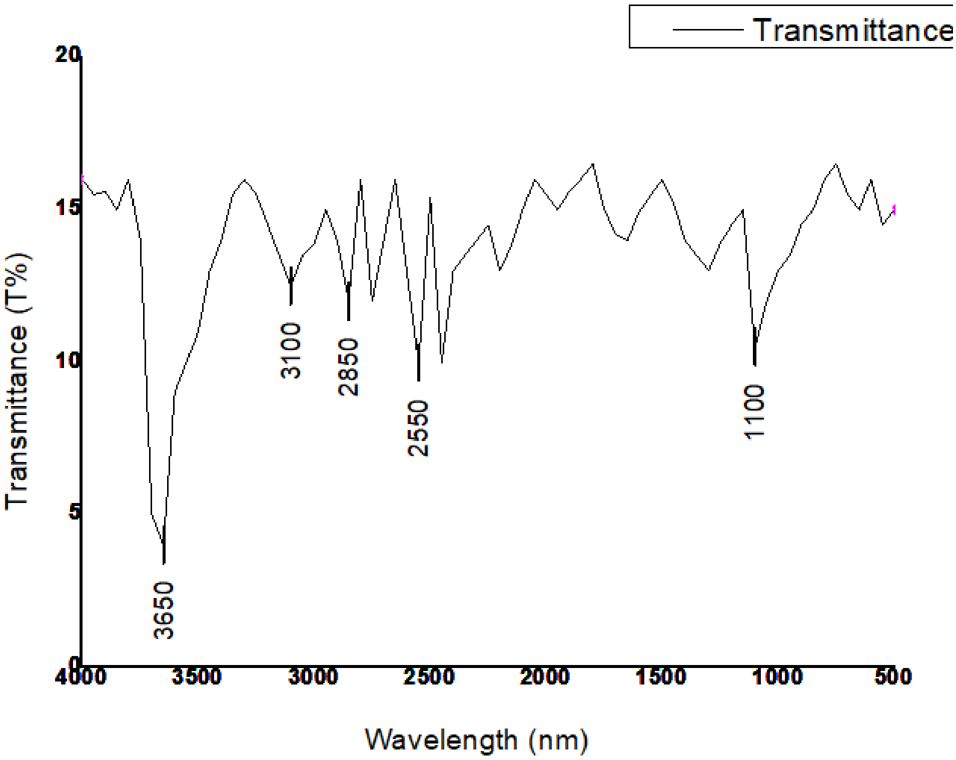

Figure 5: FTIR spectrum of different types of polymers include PE, Nylon, PS, and PVC found in *Babyloniaspirata*.

Discussion

Human activity is the primary source of microplastics in aquatic ecosystems. This activity includes high levels of anthropogenic fishing, direct industrial effluent emissions, home wastewater disposal, riverine discharge carrying leftover waste from neighboring tourists, abandoned artificial fishing gear that was mostly used for farming shrimp and fishing, and so on. The primary causes of the contamination in coastal microplastics were land-based plastic trash that washed ashore and intensive fishing (Abisha *et al.*, 2024).

The study area is widely contaminated with microplastics, according to the current research. The microplastic abundance at Stations 1, 2, 4, and 5 was identified as a result of nearby tourism, abandoned synthetic fishing gear that was extensively employed for local fishing, and residential effluents. The primary causes of the coastal microplastic pollution at these stations which are a reflection of the heavy fishing activity, were land-based plastics that were swept over and included beach trash and plastics carried by currents from other coastal locations.

The average amount of microplastics discovered in the gastropods during this investigation was higher than the amount discovered during other researchers (Li *et al.*, 2018; Dowarah *et al.*, 2019, 2020; Sathish *et al*., 2020; Patterson *et al.*, 2021).

The most traded Babylon mollusk worldwide is the ivory shell (*Babylonia spirata*) (Moazzam and Ahmed, 1994; Mohan, 2007; Tan and Low, 2013). Commercial fishing is conducted throughout nearly the whole range of *B. spirata*; however, Pakistan and India are the principal fishing nations (Ayyakkannu, 1994). All year long, fishing is done for *B. spirata*, particularly in the Indus Delta. *B. spirata* is regarded as a significant seafood that is mostly consumed in Taiwan, Japan, China, Thailand, and other Southeast Asian countries. (Moazzam and Moazzam, 2023).

More precisely, the sediment microplastics were reflected by the bottom scavenger *B. spirata* (Abisha *et al.*, 2024). *B. spirata* were found to have higher fiber occurrences. This could be because fibers are ejected more slowly from these organisms (De Witte *et al*., 2014; Li *et al.*, 2019; Patterson *et al.*, 2021) and are more difficult for organisms to remove once they become trapped in their gills and hepato-pancreas, which causes the fibers to be retained for extended periods of time (Renzi *et al*., 2018). According to previous research (Browne *et al.*, 2011; Van Cauwenberghe *et al.*, 2013; Stolte *et al.*, 2015; Graca *et al.*, 2017; Jeyasanta *et al*., 2020), these observations were identical. The prevalence of fibers could be brought on by an increase in laundry output, fishing activity, the decomposition of fishing gear, and the release of unprocessed sewage (Napper and Thomson, 2016; Wang *et al.*, 2019). According to Zaki *et al.* (2021), gastropods consume dark hues including blue, brown, and black. The most prevalent and widespread color of microplastics found in marine invertebrates is black (Hasbudin *et al*., 2022). In the present study, black fiber was dominated, the similar result also was reported by Zaki *et al.* (2021) in gastropods of the Malyasian estuary. Tahir *et al*. (2019) identified blue and black as the two dominant colors in their investigation. Tire abrasion may have caused the black microplastics (Wik and Dave, 2009). Microplastics may have become transparent fragments or films due to weathering and environmental degradation, causing them to lose their color (Duis and Coors, 2016; Patterson *et al.*, 2021). The red and blue microplastics may have originated from synthetic materials like ropes, lines, and floating buoys that are utilized in fishing and similar activities (Patterson *et al.*, 2021).

On the other hand, fragments have been found to be the second most common kind. The disintegration or weathering of bigger, scattered polymers could be the source of these fragments. The preponderance of fragments suggests that macroplastics or litter are more common in the area under study. The frequency of films in *B. spirata* was, however, lower than in those. This may be because the films' small mouths caused them to reject or avoid ingestion due to their structural makeup, which is wider than fibers and longer than pieces. Microbeads were the least frequent form discovered in the samples. Typically, microbeads fall into the size range of less than 100 µm and are produced as principal microplastics for use in industry and cosmetics. It is acknowledged that the size, shape, and surface characteristics of microplastics may influence their favored rejection and ingestion, even if the precise particle selection processes of *B. spirata* are still being studied (Ward *et al*., 2019).

Similarly, the size groups 500–1000 µm and >1000 µm were predominant microplastics; however, at stations 1 and 4, the proportion of <100 µm sized microplastics was higher. An initial understanding of how the environment and related elements, such as hydrodynamic forces (Vermeiren *et al*., 2016; Krelling *et al*., 2017; Saha *et al*., 2021).

To determine the link between the abundance of microplastics (microplastics/ind and microplastics/g of tissue) and the various morphometric characters (soft tissue weight, total weight, and total length,) of the *B. spirata* under study, a comparative analysis was carried out. Different morphometrics and Microplastics abundance in *B. spirata* exhibited a positive association, suggesting that the quantity of microplastics absorbed per gram of soft tissue in gastropods rises with size. The microplastics increase, the contamination level, and the properties of microplastics present in these places all significantly affect the microplastic abundance in the organisms. According to Abisha *et al*. (2024) *B. spirata* had the highest microplastics items/ind, presumably as a result of their larger size, greater retention capacity, and detritivore eating habits.

In *B. spirata* (<50 mm and >50 mm), no significant difference (p>0.05) was found; this may be due to the small size differences in the samples of specimens at different even locations that were obtained. Future research is necessary to comprehend how an organism's size affects its intake of microplastic, though.

Using a scavenging-feeding tactic is less discerning in terms of particle size, color, and shape than predation (Rummel *et al*., 2016). More research is nevertheless required in this area because the environment, mollusk feeding habits, availability, chemical makeup, retention duration, microplastic adsorption potential, and the leaching and mimicking qualities of additives used during polymerization can all have a substantial impact on the abundance of microplastics in gastropods.

FTIR is used to identify polymers by generating spectra that may be compared to spectral libraries that represent the material's many chemical functions (Zarfl, 2019). The majority of the persistent plastics, such as PE and nylon, have been released during fishing activities and have ended up in aquatic environments. In addition, they are utilized in face washes, industrial insulation, and packaging

(Browne *et al.,* 2011; Gurjar *et al.,* 2023).This may have made it easier for gastropods to consume these polymers in bottom-dwelling deposits. PVC is a material that is widely used in household products since it is inexpensive, strong, flexible, elastic, and simple to assemble. Furthermore, PVC is widely used in electrical installations, roofing materials, clothing, and pipelines (Bancin *et al.,* 2019). PS is a type of plastic that is produced by polymerizing styrene monomers. According to Hariady *et al*. (2014), PS is cheap, translucent, stiff, brittle, and incredibly lightweight. PS is used for food packaging, such as Styrofoam (Kholidah *et al.,* 2018).

Conclusions

Microplastics were successfully extracted from all samples of *B. spirat*a, demonstrating the extent of microplastic pollution in the research area. These findings highlight the need for quick action to monitor and minimize plastic and microplastic pollution to lessen the possiblehazards to the environment, related biota, and human populations that microplastics could pose. *B. spirata* have a favorable relationship with their surroundings and are valuable bio-indicators of microplastic. Given that the current study offers reference data regarding the existing state of microplastic contamination, the gastropods can serve as bio-indicators for the purpose of analyzing microplastics in the database for subsequent monitoring. More research is needed to fully comprehend how microplastics affect *B. spirata*, their role as environmental sentinels, how humans are exposed to microplastics through eating gastropods, and how this affects human health. These findings shed light on the specificity of swallowed microplastic in *B. spirata*, which can be utilized as a starting point for future analyses of the level of microplastic pollution in the area and how it affects the trade in mollusks.

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Conflicts of interest

The authors declare no conflict of interest.

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