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Analysis of Ingested Microplastics by the Selected Freshwater Fish

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ABSTRACT

Microplastics in water has now become a significant problem in many water bodies which in turn impacts the different organisms that live in these water bodies. The study focuses on the ingestion of microplastics by *Labeo rohita*, which is a widely distributed and economically important freshwater fish species. The study focused on the detection and quantification of microplastics in the stomach of *Labeo rohita* collected from different sites exposing different degrees of pollution. Samples were obtained from three distinct freshwater environments: The first one represents a heavily polluted pond while the other is a moderately polluted pristine reservoir, and the third one, a river. The captured fish were examined and dissected using the light microscopy and Fourier-transform infrared (FTIR) spectroscopy for detection and enumeration of microplastics. Results showed that fish collected from the highly polluted pond contained the highest levels of microplastic with fibers and fragments being the most abundant. The fish collected from the moderately polluted pristine reservoir contained less microplastic particles, and those found in the river contained negligible amounts of contamination. Subsequent characterization of the findings indicated that microplastics are present in different organs such as the stomach and the intestines, which could point to the existence of bioaccumulation and possible physiological impacts. This paper also established the presence of microplastic pollution in *Labeo rohita* and calls for specific pollution control measures since existing measures have insufficient effects in controlling environmental pollutants.

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INTRODUCTION

Since the initial synthesis of plastics in the early 1900s, the industry has been a worldwide success (Piringer and Baner 2008). Plastic is lightweight, durable, and resistant to certain chemicals, its use in goods has grown throughout the last ten years (Hopewell et al., 2009; Sharma et al., 2017). Most synthetic polymers that have been effectively developed to replace materials like metal, glass, ceramic, and wood items (Wong et al., 2015). The extensive usage of plastics has grown to be a major source of pollution and is considered a global problem of special concern (Su et al., 2016). According to a prior survey, 273.27 million tons of plastic garbage were produced worldwide year in 2010 (Jambeck et al., 2015). According to reports, China produces approximately 60 million tons of plastic garbage annually, making it the world's greatest producer (Jambeck et al., 2015; Qu et al., 2019). In the meanwhile, the top 3 high-income nations that produced plastic garbage were the USA (37.8 million tons), Germany (14.4 million tons), and Japan (7.99 million tons). With 5.046 million tons, Indonesia led the Southeast Asian area in the production of plastic garbage, followed by Thailand (3.533 million tons) and Vietnam (3.268 million tons). Malaysia produced an exceptional quantity of plastic garbage, placing it

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seventh among Southeast Asian nations.When referring to tiny plastic trash that is less than 5 mm, the term “microplastics” was first used in 2004. The scientific community later came to adopt the phrase, and it is now often used widely in scholarly publications (Van Cauwenberghe et al., 2015). They are often divided into two categories: primary and secondary microplastics. Microbeads and raw plastic resin are examples of primary microplastics (Hernandez et al., 2017 Everyone was unanimous that rivers play a significant role in moving most of the plastic waste from the land into the marine environment. According to prior research, between 1.15 and 2.41 million tons of plastic debris are dumped into the ocean each year (Lebreton et al., 2017). According to their assessment, 122 contaminated rivers were responsible for almost 90% of all plastic inputs. The first data that freshwater fish consume microplastics was discovered in 2014. Twelve percent of the wild gudgeons (*Gobio gobio*) they studied, which were collected in eleven different French streams with different environmental stresses, had microplastics in their digestive tracts (Sanchez et al., 2014). Additionally, Phillips and Bonner reported that fish in freshwater drainages in the Gulf of Mexico sometimes consumed plastic, with an 8% incidence of microplastic ingestion (Phillips and Bonner, 2015). Subsequently, Peters and Bratton discovered that in the Central Brazos River Basin of Texas, 45% of the fish studied had consumed microplastic (Peters and Bratton, 2016). In a more recent study, Jabeen discovered microplastics in 95.7% of freshwater fish after studying micro- and mesoplastic contamination in Chinese freshwater and marine fish (Jabeen et al., 2017). Additionally, they revealed for the first time the prevalence of plastics in the intestines, indicating that in some fish species, the number of plastics in the intestines was much greater than in the stomachs. Silva-Cavalcanti evaluated the consumption of microplastic by Hoplosternum littorale, a popular freshwater fish that people eat on a regular basis in Northeast Brazil, in the same issue. Fish from a city-crossing segment of the Pajeú River were taken at four sample locations, and it was discovered that 83% of the fish had microplastic in their digestive systems. This percentage is far higher than what has been recorded for other freshwater, estuarine, or even marine species (Cavalcanti et al., 2017).

Why *Labeo rohita*?

Rohu is actually a popular freshwater- consumable fish in Asia particularly South Asian regions namely India, Bangladesh, Nepal, and Pakistan. Several factors contribute to its popularity: Several factors contribute to its popularity:

1. Availability: Rohu is found all over the Indian sub-continent inhabiting in fresh water like rivers, lakes, and reservoirs. It is endogenous to Rivers including, Ganga, Yamuna ,Brahamaputra and their affluents. This makes it easily available to the local people and as such widely utilized (Hossain et al., 2022; Majumder and Saikia, 2020).

2. Cultural Preference: Golds of Rohu are in vogue in South Asian food. It’s used in many of the regional meals and their preferred choice because of its taste and texture (Das et al., 2013).

3. Nutritional Value: Rohu provides proteins, essential fats, vitamins, and minerals to the consumer and can also help augment their immune system. Also, it is recognized as a nutrient food product, thus facilitating its consumption as an edible fish (Zaman et al., 2024; Subasinghe et al., 2001).

4. Versatility: Rohu can be cooked by fry, grill, curry and steam. It also gets preference in many recipes because of the variety of ways it can be cooked.

5. Economic Importance: Farming of Rohu has taken a pace and it forms a separate industry in some places. This made Rohu commercially farmable giving people a new source of income thus making the species available in the market (Mridul et al., 2024; Bagchi and Jha, 2011).

MATERIALS AND METHODS

Study site and sample collection

A study was conducted in Gujarat, encompassing various freshwater habitats, including a Pond (Malav Talav (Dholka)-Figure 1a), a Dam (Dharoi Dam-Figure 1b), and a River (Sabarmati River-Figure 1c). All three sites experience the impacts of various human activities including urbanization that is through expansion of concrete structures for habitation, farming and industrial activities that favor economic growth of a nation. These water bodies can get inputs of microplastics from the urban wastewater discharge, industrial waste discharge, and agricultural drainage water amongst others, thus are suitable for investigation. At each site, sixty samples were taken, and each one was transported to the laboratory in order to be examined in detail in this study. Employing standardized methods for gathering samples and recording environmental factors are very beneficial in enhancing the precision and accuracy of diverse data gathered from several sampling locations. Additionally, the fish specimens should be caught using techniques that are gentle enough to prevent the destruction of microplastics in their stomachs. Employing suitable techniques to get specimens that are typical of a range of fish species, sizes, and ages enhances the representativeness of the samples gathered. Additionally, in order to identify the sample and preserve it, it must be properly labeled with specific information and stored in clean, airtight containers to prevent deterioration during storage and transit. Sample variation that might influence findings can be minimized by swiftly transferring the sample to the lab and keeping it in a cold atmosphere. To improve safety and advance ethical standards, researchers and other users of human subjects should adhere to safety precautions, safety laws and regulations, and the appropriate use of protective equipment.

Extraction and characterization of microplastics

After systematically gathering fish from three distinct locations, namely Dharoi Dam, Sabarmati River, and Malav Talav (Dholka) each characterized by its unique freshwater ecosystem, meticulously procure 60 samples from each site, ensuring a representative sample size, with the primary species of interest being *Labeo rohita*, commonly known as Rohu, renowned for its predilection for freshwater habitats, following which each sample undergoes precise dissection to extract the gut and determine biomass, which is subsequently subjected to chemical digestion to dissolve organic matter, thereby facilitating comprehensive analysis under stereomicroscopes, enabling detailed observation and examination of the specimens.

Upon the catch of certain fish, the stomach and small intestine have been removed from the

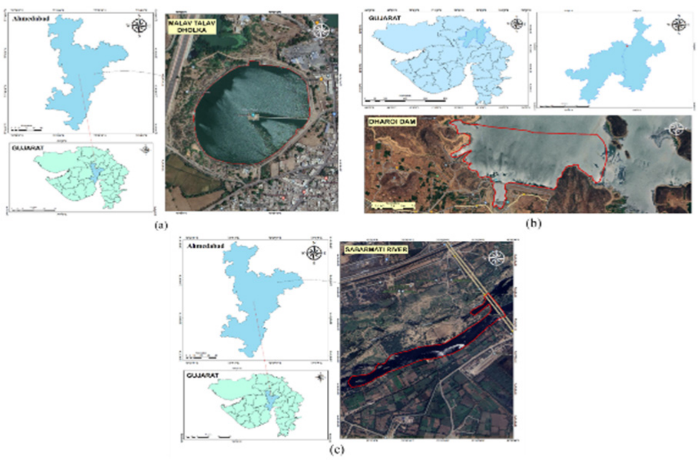

Fig. 1. Study sites (Arc GIS maps) (a) Malav Talav (Dholka), (b) Dharoi Dam, (c)Sabarmati River

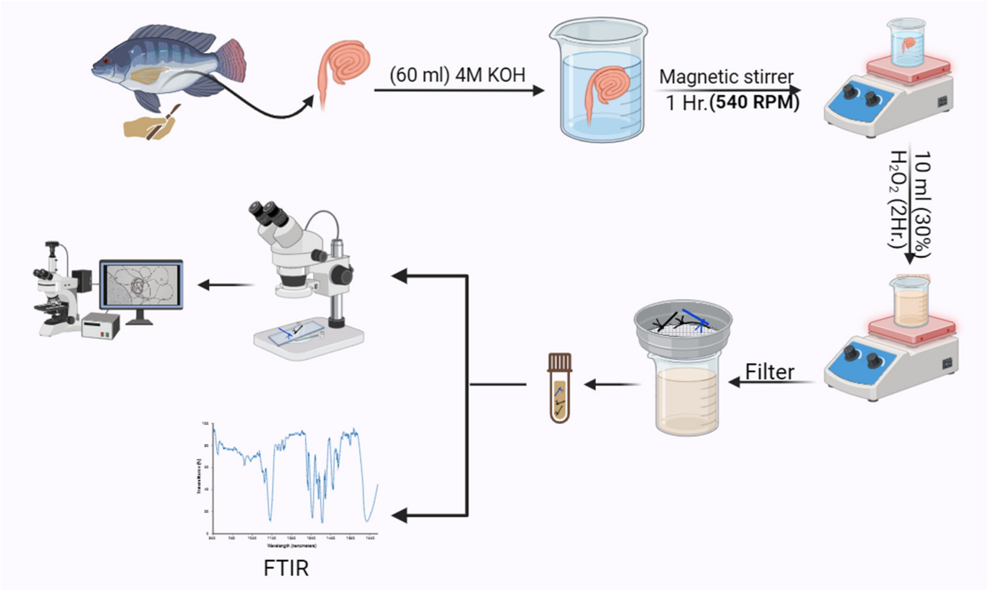

Fig. 2. Flowchart of methodology

sacrifices. Both the mass of the digestive tract without the contents and its wet mass with the contents were weighed in order to determine the wet weight of the contents. These methods include chemical digestion with 60 ml of 22%, density gradient centrifugation, and enzymatic digestion. In order to test the filter's and the particles' ability to dissolve in the 4% potassium hydroxide (KOH) solution and the 30% hydrogen peroxide (H_2O_2) solution, all subsequent steps of the dissolution process were carried out in a magnetic stirrer at 560 RPM for one hour each (Dawson et al., 2020). The identification of microplastic is done by visual observation with the help of a Nikon Microscope (Stereo Zoom SMZ-10, Japan), and morphometric study is done using a Moticam 1080 HDMI and USB camera and a Lawrence and Mayo compound microscope, photomicrographs of the samples were taken utilizing the program Motic Images Plus 3.0 (x64), measurements were taken (Chauhan et al., 2023).

ATR-FTIR analysis

After the microplastics were morphometrically classified, the next phase in the study process was a chemical analysis using micro-ATR-FTIR techniques, which are thought to be the most effective test for determining the polymer type (Jung et al., 2018). It was proposed that the functional groups of the sample may be used employing ATR-FTIR to identify between different types of plastic. Certain plastics emit particular infrared spectra due to unique bond configurations, which in turn distinguishes the material's composition. Wavelengths in the spectral range of 500 to 4000 cm^{-1} were used for the measurements. Merely 10% of samples belonging to each main class of microplastic material type were chosen for FTIR scanning in order to analyze their chemical makeup (Daniel et al., 2020). Essential ATR-FTIR software was used for noise clearance and visual identification was performed as described by (Jung et al., 2018). Surface degradation and biofouling may reduce the degree of match spectra to normal (Tiwari et al., 2019).

RESULTS AND DISCUSSION

Microplastic abundance

In the present study, MP contamination was assessed in fish, *Labeo rohita*, where a total of 180 fish were examined for Microplastics, and all 180 specimens had contamination with

Microplastics. The study investigated microplastic contamination in the gut tissues of *Labeo rohita* from three freshwater reservoir freshwater habitats, including a Malav Talav (Dholka), a Dharoi Dam, a Sabarmati River. A total of 5937 microplastics were recorded from the gut of fish, out of which Malav Talav (Dholka) has total 2444, Dharoi Dam has total 1838 and a Sabarmati River has 1655. The average abundance of microplastics contamination was recorded in Malav Talav (Dholka) is 40.73 ± 2.22 microplastics/individual, in Dharoi Dam 30.63 ± 2.10 microplastics/individual, in a Sabarmati River 27.58 ± 2.13 microplastics/individual. Also, average abundance of microplastics contamination was recorded in Malav Talav (Dholka) is 26.124 ± 7.39 microplastics/Kg, in Dharoi Dam 19.532 ± 3.87 microplastics/Kg, in a Sabarmati River 17.478 ± 4.23 microplastics/Kg. The microplastic contamination was recorded highest in Malav Talav (Dholka), while less in Sabarmati River.

The most prevalent types of microplastics identified were Threads, Films and Fragments (Table 1) with sizes ranging from microscopic to macroscopic dimensions.

ATR-FTIR analysis

In the context of microplastics, Fourier-transform infrared spectroscopy (FTIR) results often include the number of peaks in the sample spectrum to identify the kind of plastics. FTIR is used to distinguish between various types of plastics as each one emits a unique infrared spectrum. Results of analysis of test is given below.

1. Malav Talav (Dholka): The data provided includes plastics identified by their wavenumber (measured in cm^{-1}), and intensity, along with the types of microplastics associated with each peak. For instance, peak number 10 corresponds to a wavenumber of approximately 1636.30131 cm^{-1} and an intensity of 0.16771, indicating the presence of Polycarbonate (PC) microplastics. Similarly, peak number 19, with a wavenumber of around 2264.35774 cm^{-1} and an intensity of 0.92634, is associated with Polyacrylonitrile (PAN) microplastics. Peak number 22 represents Polyvinyl Chloride (PVC) microplastics, showing a wavenumber of about 2853.27711 cm^{-1} and an intensity of 0.76200. Peaks 23 and 24 exhibit multiple types of microplastics: peak 23 is linked to Polypropylene (PP), Polystyrene (PS) microplastics, while peak 24 is associated with, Polyethyleneimine (PEI), and Polyurethane (PU) microplastics. This data aids in the identification of different microplastic types present in samples.

2. Dharoi Dam: A peak at wavenumber 1636.30131 cm^{-1} was attributed to polycarbonate (PC), while another peak at 2273.70935 cm^{-1} indicated the presence of polyacrylonitrile (PAN). Additionally, a peak at 3300.60074 cm^{-1} was associated with polyurethane (PU) and nylon (PA).

3. Sabarmati River: The analysis of microplastics through infrared spectroscopy reveals distinct spectral signatures associated with different polymer types. Within the dataset examined, peak number 1, detected at a wavenumber of 1636.45 cm^{-1}, signifies the presence of polycarbonate (PC). Peak number 2, observed at 3324.66 cm^{-1}, is indicative of polyurethane (PU), while also possibly suggesting the presence of epoxy resin (EP).

Microplastics are accumulated in various freshwater bodies by agricultural irrigation

Table 1. Abundance of microplastic contamination in *Labeo rohita* in Gujarat, all values are in (Mean ± SD).

Type	Dharoi Dam	Malav Talav (Dholka)	Sabarmati River
Black Threads	12.285 ± 1.78	15.819 ± 2.17	9.021 ± 2.04
Blue Threads	3.058 ± 1.46	4.785 ± 1.26	3.333 ± 1.06
Transparent Threads	7.201 ± 1.53	9.688 ± 1.58	6.715 ± 1.25
Red Threads	2.173 ± 1.15	3.267 ± 1.26	2.923 ± 1.25
Blue Fragments	1.99 ± 0.96	2.984 ± 1.17	2.145 ± 0.85
Red Fragments	0.881 ± 0.65	0.989 ± 0.58	0.871 ± 0.57
Films	1.542 ± 0.74	2.016 ± 0.72	1.261 ± 0.76
Average Microplastic/Individual	30.633 ± 2.10	40.73 ± 2.22	27.583 ± 2.13
Average Microplastic/Kg	19.532 ± 3.87	26.124 ± 7.39	17.478 ± 4.23

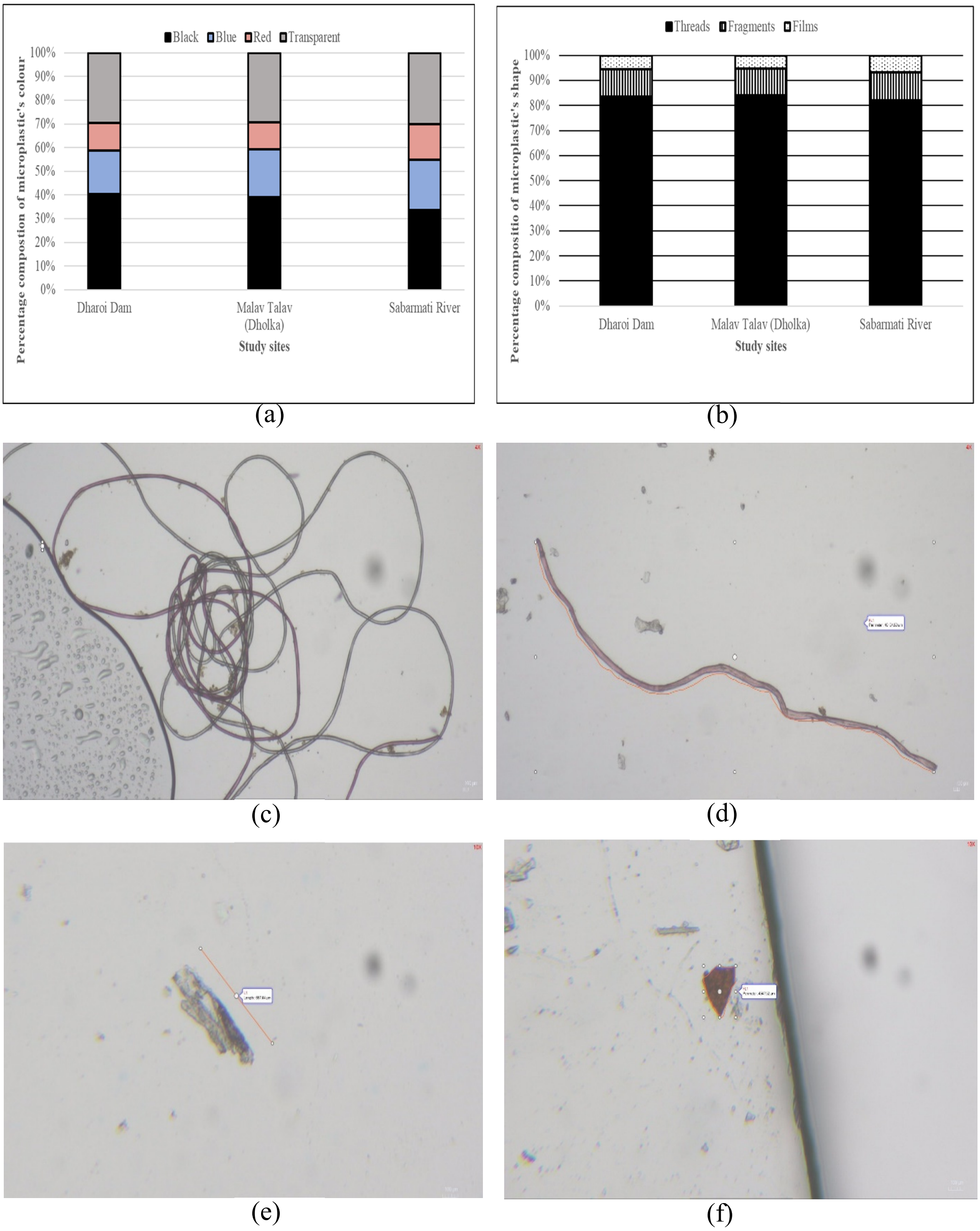

Fig. 3. (a) Showing percentage composition of microplastic's color, (b) Showing percentage composition of microplastic's shape, (c) and (d) Microplastic thread, (e) Microplastic film, (f) Microplastic fragment.

and outflow from urban areas and industrial operations. This suggests that the amount of microplastics entering ponds, rivers, and dams may vary depending on how far they are from populous areas, what kinds of activities are done nearby, and how quickly the water bodies empty (Eriksen et al., 2013). Determining the origins and patterns of microplastic dispersion in freshwater systems may be accomplished by observing the concentration of microplastics in

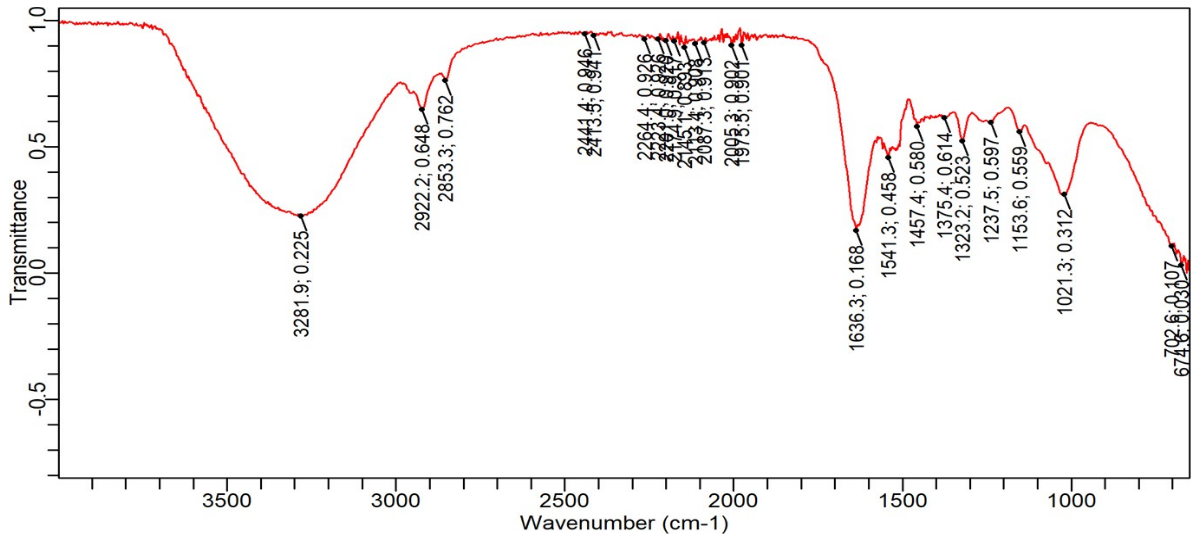

Fig. 4. IR Graph of Malav Talav (Dholka).

Table 2. Types of plastics according to IR Graph peaks (Malav Talav-Dholka).

Peak Number	Wavenumber (cm^{-1})	Intensity	Types of plastics
10	1636.30131	0.16771	Polycarbonate (PC)
19	2264.35774	0.92634	Polyacrylonitrile (PAN)
22	2853.27711	0.76200	Polyvinyl Chloride (PVC)
23	2922.23286	0.64755	Polypropylene (PP) Polystyrene (PS)
24	3281.92097	0.22524	Polyethyleneimine (PEI) Polyurethane (PU)

fish taken from various settings. According to this research, there are differences across ponds, rivers, and dams in some physical and chemical aspects that affect the destiny of microplastics (Su et al., 2016; Watkins et al., 2019). As an instance, whereas rapid river systems have the potential to carry microplastics downstream, still water systems like ponds may promote the settling of microplastics. Dams have the potential to hold onto sediments and microplastics, which alters the materials' availability to aquatic life (Su et al., 2016; Watkins et al., 2019). Corresponding with these results, we found that during the current investigation, fish ingestion of microplastic was greatest in Malav Talav (Dholka), followed by Dharoi Dam, and lowest in Sabarmati River. One can forecast how habitat characteristics influence the buildup, ingestion, and transmission of microplastics throughout freshwater food webs by researching fish in a variety of environments.

Microplastics are often mistaken for food and ingested by fish, leading to their accumulation in the gastrointestinal tracts (Slootmaekers et al., 2019). From research point of view, assessment of gut content of a dominant fish species will act as an indicator of the pollution levels within the water bodies they inhabit. So, our study employed gut content assessments of *Labio rohita* to evaluate the extent of microplastic contamination in three freshwater bodies (Malav Talav (Dholka), Dharoi Dam and city stretch of Sabarmati River) in and around the city of Ahmedabad in Gujarat. Results indicated that Malav Talav (Dholka) with 40.73 ± 2.22 microplastic/individual has highly contaminated with Microplastics, while Sabarmati River 27.583 ± 2.13 microplastic/individual and Dharoi Dam with 30.633 ± 2.10 microplastic/individual have comparatively less contamination levels. As for type of Microplastics are concerned the result of study indicate that filaments top with 84.061% while films represent only 5.206% of Microplastics contamination

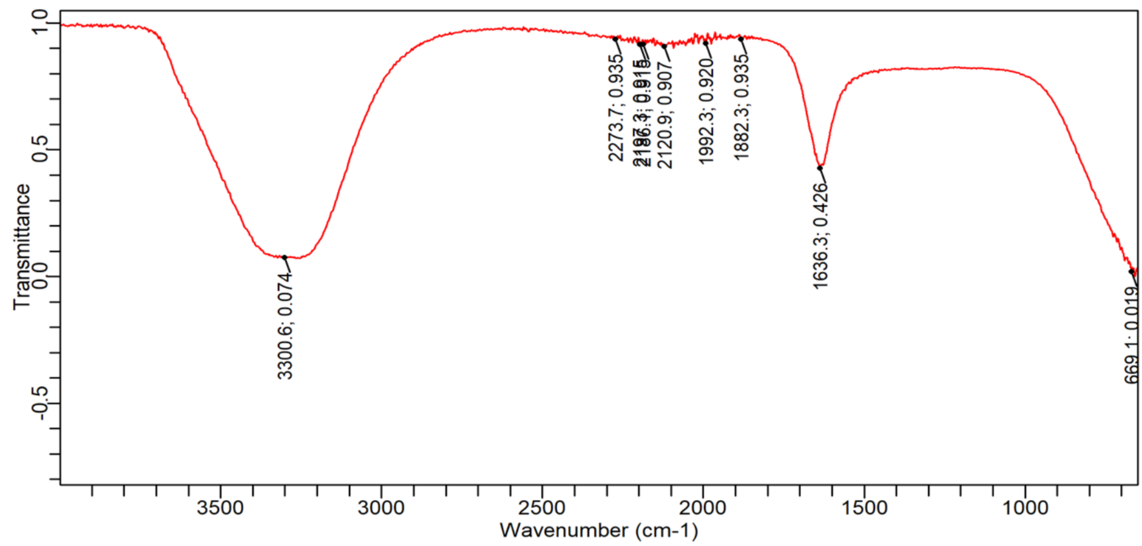

Fig. 5. IR Graph of Malav Talav (Dharoi Dam).

Table 3. Types of plastics according to IR Graph peaks (Dharoi Dam).

Peak Number	Wavenumber (cm^{-1})	Types of plastics
1	1636.45	Polycarbonate (PC)
2	3324.66	Polyurethane (PU) Epoxy Resin (EP)

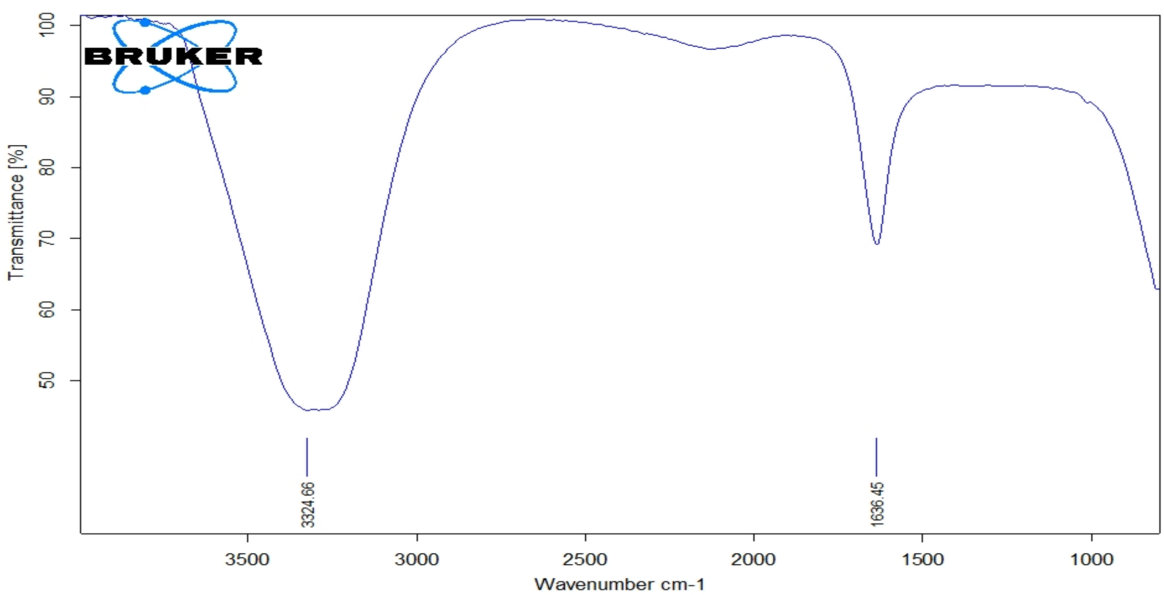

Fig. 6. IR Graph of Sabarmati River.

Table 4. Types of plastics according to IR Graph peaks (Sabarmati River).

Peak Number	Wavenumber (cm^{-1})	Types of plastics
2	1636.30131	Polycarbonate (PC)
8	2273.70935	Polyacrylonitrile (PAN)
9	3300.60074	Polyurethane (PU) Nylon (PA)

across the three sites.

ATR-FTIR analysis conducted to assess the chemical nature of microplastics in Malav Talav (Dholka) identified seven types of microplastics: Polycarbonate (PC), Polyacrylonitrile (PAN), Polyvinyl Chloride (PVC), Polypropylene (PP), Polystyrene (PS), Polyethyleneimine (PEI), and Polyurethane (PU). In Dharoi Dam, the study found four types of microplastics: polycarbonate (PC), polyacrylonitrile (PAN), polyurethane (PU) and nylon (PA) while in Sabarmati River, only three types- polycarbonate (PC), polyurethane (PU) and epoxy resin (EP) were identified.

To contextualize our findings, we compared our data with existing reports on microplastic contamination from other studies conducted within the country and in neighboring regions.

The Sabarmati River, Dharoi Dam, and Malav Tadav are contaminated by microplastics. These little pieces of plastic originate from several sources. Plastic waste from cities is dumped into parks, streets, and rivers. This trash washes into rivers and lakes during rainy seasons. Plastic debris is also released by factories close to water. Plastic is used on farms for items like sacks of fertilizer, pipelines, and mulch. These may decompose and wind up in the water close by. Things become worse when people litter or forget to put away their rubbish. People throw plastic around reservoirs and along riverbanks. All of this poses a significant issue for these freshwater areas. Over time, when farmers irrigate their crops heavily or when it rains a lot, the plastic accumulates (Sarijan et al., 2019). The combined effect of these human-caused activities may cause microplastics to disperse widely across the Sabarmati River, Dharoi Dam, and Malav Tadav. This affects the ecosystem, endangering aquatic species and posing health risks to those who rely on these freshwater supplies. The discovery that microplastics have been found in *Labeo rohita* digestive systems sends a clear message about the consequences of

Table 5. Abundance of number of microplastic/individual.

Sr. No.	Water body	Fish species	No. of microplastic/ individual	References
1	Upper River Ganga stretch	*Labeo dero*	15.42 ± 9.33	Badola et al., 2023
2	Xiangxi River, China	*Labeo rohita*	1.5 ± 1.38	Zhang et al., 2017; Kumar, et al., 2021
3	Pearl River, China	*Labeo rohita*	23.8 ± 7.0	Zheng et al., 2019; Kumar, et al., 2021
4	Chi River, Thailand	*Labeo rohita*	1.76 ± 0.97	Kasamesiri and Thaimuangphol, 2020; Kumar, et al., 2021
5	Ulhas River Estuary	mudskipper fish	3.75 ± 6.11	Verma et al., 2022
6	Ravi River (Balloki Barrage), Pakistan	*Labeo calbasu, Cirrhinus mrigala*	33.07 ± 23.11	Tariq et al., 2022
7	Mahanadi River, India	*Various sp.*	14.7 ± 3.7	Ganie et al., 2024
8	Ravi River (Sidhnai barrages), Pakistan	*Labeo calbasu, Cirrhinus mrigala*	28.1 ± 20.7	Aslam et al., 2023
9	Bhogdoi River, a tributary of River, India	*Labeo rohita*	12.11 ± 2.13	Ahmed et al., 2023
10	The Ganga (Patna), India	*Labeo rohita, Wallago attu and Mystus tengara*	5.21 ± 2.51	Kumari et al., 2023
11	The Ganga (Uttarakhand), India	*Labeo dero*	15.42 ± 9.33	Badola et al., 2023
12	Alaknanda River (Garhwal), India	*Various sp*	12.66 ± 7.73	Bhatt et al., 2023
13	Kollidam and Vellar Rivers, India	*Chanos, Chanda* and *Gerrus sps*	22.8 ± 4.9	Anandhan et al., 2022
14	Malav Talav (Dholka), India	*Labeo rohita*	40.73 ± 2.22	Present Study
15	Dharoi Dam, India	*Labeo rohita*	30.633 ± 2.10	Present Study
16	Sabarmati River (Ahmedabad), India	*Labeo rohita*	27.583 ± 2.13	Present Study

plastic pollution that flow downstream from their initial origins. These substances are derived from materials used in industries and consumer goods, which not only harm marine life but also serve as a warning that nearly all *Labeo rohita* have been contaminated by these harmful substances. These substances spread poisons through streams, increasing the likelihood that plastic litter will harm the environment.

Aquatic life and humans that rely on freshwater sources are both impacted by microplastic contamination in freshwater environments. Examining fish from ponds, rivers, and dams for microplastics helps us determine the extent of the pollution, the environmental concerns it presents, and the potential routes of human exposure (Gusmão et al., 2016). For these reasons, it is crucial to gather this knowledge that might assist us control the situation so that it doesn't occur again, and therefore preserve fish species populations and humans in general. To put it quickly, examining microplastic presence in freshwater fish in their varied habitats would let us understand diverse origins, distribution patterns, ecological linkages and consequently control methods of microplastic contamination in freshwater bodies. Understanding the intricacy of microplastic pollution and devising the techniques targeted at treating this prevalent ecological problem is vital.

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The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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