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Assessment of Microplastic Ingestion in Freshwater Fish Labeo Rohita From the Indus River Crossing Dera Ismail Khan

Afira Afsheen

Government Girls Degree College number 1, Dera Ismail Khan

Email: sko162665@gmail.com**Ruqia Bibi**

Professor, Government Girls Degree College number 1, Dera Ismail Khan

Nida Khalid

M.Phil Student, Department of Zoology, Khawaja Fareed University of Engineering and Information Technology

Sumbal

MPhil Student, Department of Zoology, University of Science and Technology, Bannu

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Corresponding Author:**Afira Afsheen**Email: sko162665@gmail.com

The increasing contamination of MP's in aquatic environment particularly in fresh water ecosystem has become a serious challenge for human health fresh water system like Indus river system is an important transport routes for plastic contamination however the research regarding on micro plastic ingestion in commercially impotent fish species remains limited There for the present study was aimed to investigate the ingestion of micro plastic in fresh water fish Labeo rohita on the basis of size, color and shape. A Total of 100 fish specimen were collected from three different sites of Indus River Dera Ismail Khan the three locations are named as upstream, midstream and downstream. The fish specimen were collected between November 2025 to April 2026. The Gastrointestinal Tracts of each specimen is removed after dissection for further analysis the GIT tract is digested in 30% hydrogen per oxide solution to is date organic materials the solution was incubate at 48c' and then Filters through Filter paper the filter paper contain the remaining particles was dried in a laboratory oven at 37c' After drying the filter paper was examined under a compound microscope. The extracted MPs were identified and characterized based on their size shape and color on way Anova and correlation test were carried out to determine the variance and relationship with fish biometric parameters. Results revealed that 78% of fish contained microplastics, with a sum of 156 molecules detected along with a mean profusion of 1.56 ± 1.21 items per fish. The predominant type was fibers (67%), then succeeded by films (14%), lines (10%), fragments (6%), and foam (3%). Black-colored particles were most prevalent (29%). Microplastic abundance was significantly higher at downstream sites ($p = 0.017$), and showed positive correlations with body length ($R^2 = 0.41$), body weight ($R^2 = 0.46$), and gut weight ($R^2 = 0.39$). *Labeo rohita* from the Indus River are significantly exposed to microplastic contamination. It is recommended that continuous monitoring, enhanced management of garbage practices, and consciousness of the public programs be implemented to reduce microplastic waste in freshwater habitat.

Introduction

Consuming freshwater fish contaminated with microplastic presents a notable threat to human health. Local fishermen captured the common freshwater fish, Labeo rohita, in the Dera Ismail Khan Indus River and examined the micro plastics' size.

Labeo rohita, also referred to rohu, is a key freshwater fish species found in Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan. With a slightly arched head and characteristic reddish fins, Rohu has a sleek, silver-scaled body. The maximum weight and length for adults are 45 kilograms and 2 meters, respectively. Rohu is extensively spread in tropical and temperate regions and can be found in Dera Ismail Khan's rivers, lakes, and ponds. Freshwater ecosystems are among the many aquatic habitats in which it flourishes. An omnivore, Rohu has distinct dietary preferences at different phases of life. It mostly consumes zooplankton in its early stages, but as it matures, it begins to favor more phytoplankton and submerged vegetation. (Lusher, Hollman, & Mendoza-Hill, 2017) Freshwater fish consumption of microplastics has turned into a major environmental problem that presents a covert danger to aquatic ecosystem biodiversity globally. Dera Ismail Khan, district, located in Pakistan's Khyber Pakhtunkhwa Province, contains diverse freshwater bodies including lakes, river, and streams. Which sustain a broad variety of aquatic life. However, concerns have been expressed over the possible effects of the growing amount of microplastics in these ecosystems on fish populations, human health, and the region's general biodiversity. (Khan & Setu, 2022) One major issue facing the environment is plastic pollution. Long polymer chains make up plastics. The polymerization and condensation reactions create polymers; for example, the polymerization step creates polyethylene. In addition to being insulators, plastics can be strong, lightweight, flexible, affordable, and waterproof. While some of them are not biodegradable, others can be broken down by hydrolysis, microbial activity, or ultraviolet (UV). MPs are divided into three primary groups according to their size.; microplastic measuring 25 millimeters or larger, Meso plastic ranging from 5mm to under 25mm, and micro plastic between 1mm to 5 mm. Due to their inherent hydrophobicity, microplastic reach freshwater system through atmospheric deposition, surface runoff, discharge from both treated and untreated sewage, industrial wastewater, and waste plastic debris. These particles have been detected in drinking water, alcoholic beverages, food items, and the atmosphere. Owing to their practical application, durability and resistance to breakdown, they persist in the environment and are susceptible to ingestion by various organisms..(Bhardwaj, Rath, Yadav, & Gupta, 2024). Plastic pollution is mostly affecting the 3,200-kilometer-long and economically significant Indus. Littering is seriously harming the surrounding habitats and water ecosystem (Bhardwaj et al., 2024) Single-use plastics (SUP), such as straws, plastic bags, and water bottles, are those that are discarded after a single use and frequently disposed of in rivers. The Indus has gained reputation for having the second-highest percentage of plastic pollution, accounting for an alarming 40% of all solid debris collected in the river and along its banks. In order to encourage swift action for the sustainability of the river and its tributaries, the World Bank spearheaded the study to monitor the amount and types of plastic garbage in the Indus. Over 90% of the plastic debris from the Upper Indus Basin sample sites ends up in the river. Many different types of contaminants are present in freshwater environments, most of which are linked to human activity. Furthermore, the diversification of industrial resources, changing human lifestyles, and technology improvements are all causing pollutants to change and evolve. Pollution continues to spread because of the low recycling rate and the lengthy natural disintegration period of plastics, the latter of which is still little understood. (Erdoğan, 2025) So far, five research have looked into the presence as tiny plastic particles in freshwater fish, even though knowledge about marine micro plastic grown significantly. The initial proof that freshwater fish consume micro plastic was provided by Sanchez in an initial field study released in 2014. Micro plastic were found in the gut of 12% of gudgeons in the wild (*Gobio gobio*) collected from Eleven streams in French exposed to different environment stressors. Jabeen discovered MPs 95.7% of fishes in freshwater fish in a more recent study on micro plastics pollution in freshwater and marine fish from China. Furthermore, they disclosed for the first time how common there were polymers in the gastrointestinal tract, suggesting that the quantity of plastic in the intestinal tract of certain species of fish were even higher than the stomach's. The consumption of MPs by the typical freshwater species *Hoplosternum littoralis* fish consumed on a daily foundation in Northeastern Brazil, was evaluated in the same problem. Eighty-three percent of the fish in four sample locations in a section of the Pajeú River that crosses a city. MPs within their digestive systems, which is far higher than the percentages found in other fish from freshwater, estuaries, or even the ocean. Fish have substantial nutritional and economic importance globally and are a crucial biological component of the freshwater ecosystem. Around 94% of global freshwater fisheries are located in developing countries, where they supply food and income for millions of low-income people and also contribute to the economy via exports, tourism, and recreational activities. Additionally, they produce a variety of ecological services, including controlling the dynamics of food webs and balances of nutrients, controlling flow of carbon, controlling sedimentation procedures, while serving as connections across ecosystems. Therefore, it becomes essential to safeguard freshwater fish populations against contamination from new issues like micro plastics (Pinheiro, Oliveira, & Vieira, 2017) Micro plastic negatively affect the physiological function of several organs in freshwater fishes, such as the liver, kidney, and intestines. They also cause biological changes affecting genes, immune function, reproductive systems, and behavioral patterns. When MPs adhere, they irritate the gill tissue and in return triggers for mucous secretion. Dangers of MPs ingestion (Obstruction, Inflammation and Tissue Damage) on the GIT of freshwater fish would ultimately result in a reduced metabolism of nutrient uptake(Andrade et al., 2019) Ingestion of micro plastics can cause blockage, inflammation, and tissue damage to the gastrointestinal tract of freshwater fish, which can ultimately result in a reduction in metabolism and nutrient absorption (Xue et al., 2021). *Labeo rohita*, also called rohu, is widely distributed across Indian subcontinent and occur in various freshwater habitat including lakes, rivers, and reservoirs. It is naturally found in the Indus River system as well as in other major river systems like the Ganga, Yamuna, and Brahmaputra, as well as their tributaries. *Labeo rohita* is widely thrive in fresh water

ecosystem and it is well adapted to variety of environmental conditions *Labeo rohita* is one of the most commonly consumed specie by local people (Hossain *et al.*, 2026). Its wide distribution and high market demand make it an important fish species for research purpose of investigating he Hps contamination and associated concern of human health and freshwater ecosystem. Rohu became financially viable as a result, providing individuals with a new source of money and enabling the species to be sold (Jaydipsinh, Hitesh, Ali, & George, 2025)

Material & Methodology

This study was conducted at Dera Ismail Khan, which is located in the southern region of Khyber Pakhtunkhwa, Pakistan. The Indus River is One of South Asia's principal river systems, it flows along the district's western edge and sustains a wide variety of waters fauna. Dera Ismail Khan Lies nearly roughly 230 kilometers northwest of Multan and 300 kilometers south of Peshawar. The period of sampling was November 2025–April 2026.at three selected sites along the Indus River, namely upstream, midstream, and downstream locations. These Sites were chosen according to different anthropogenic pressure levels. The research area is visible to multiple sources of plastic pollution, including municipal waste disposal, tourist activities, and domestic effluents such as detergent discharge. Plastic debris and particles were frequently observed along riverbanks and within flowing water, indicating continuous input of micro plastics into the aquatic ecosystem.



Fig.1: Sampling sites on the Indus River in D-I-Khan

Sample Collection

One hundred freshwater fish specimens in all were collected from three selected sampling locations. A minimum of 33 specimens of *Labeo rohita* were sampled from each site to ensure adequate representation. Immediately after collection, the fish specimens were placed in a clean icebox for preservation and transportation. The samples were then transferred to the laboratory of microbiology on the city campus on the same day .All collected samples were preserved at 4°C prior to further laboratory examination. All samples were processed within an appropriate time frame to avoid decomposition and ensure reliable results.

Sample Preparation and Dissection

Prior to analysis, Samples were allowed to defrost at room temperature for approximately 30 minutes. Each specimen's weight and total body length were measured and recorded. The dissection process was performed under controlled laboratory conditions using sterilized stainless steel tools.to avoid Contamination. After being properly removed, the entire gastrointestinal tract (GIT) was placed into properly labeled glass containers the excised gut was temporarily preserved in 10% formalin prior to digestion.

Hydrogen Peroxide Treatment

To maintain sterility, the preserved organs were placed in clean autoclaved glass Petri dishes for approximately 10 minutes before digestion. The gastrointestinal contents were subsequently put via chemical digestion. Almost 20 milliliters of 30%

hydrogen peroxide solution was introduced into each sample. To get rid of organic materials. The resultant solution was filtered after full digestion. Using an appropriate filter medium. The retained material on the filter was allowed to dry under covered conditions. The remaining filtered material was observed using a microscope. The collected particles of MP's were observed under compound microscope for identification and confirmation and confirmation these particles were classified on the basis of color, shape and size.

Extraction and Characterization of Micro-plastics

Fish specimen were collected from three different site of Indus river upstream midstream and downstream representing fresh water habitat a total 100 sample were collected 34 sample from upstream while 33 fish specimen were obtained from midstream and 33 from downstream the fish specimen were carefully transported in ice box to laboratory to prevent contamination each fish sample is dissected and the GIT is carefully removed and weighted the gut content were chemically digested using hydrogen peroxide to remove organic matters the processed sample were carefully examined under compound microscope and the isolated particles were categorized on the bases of color shape and size.

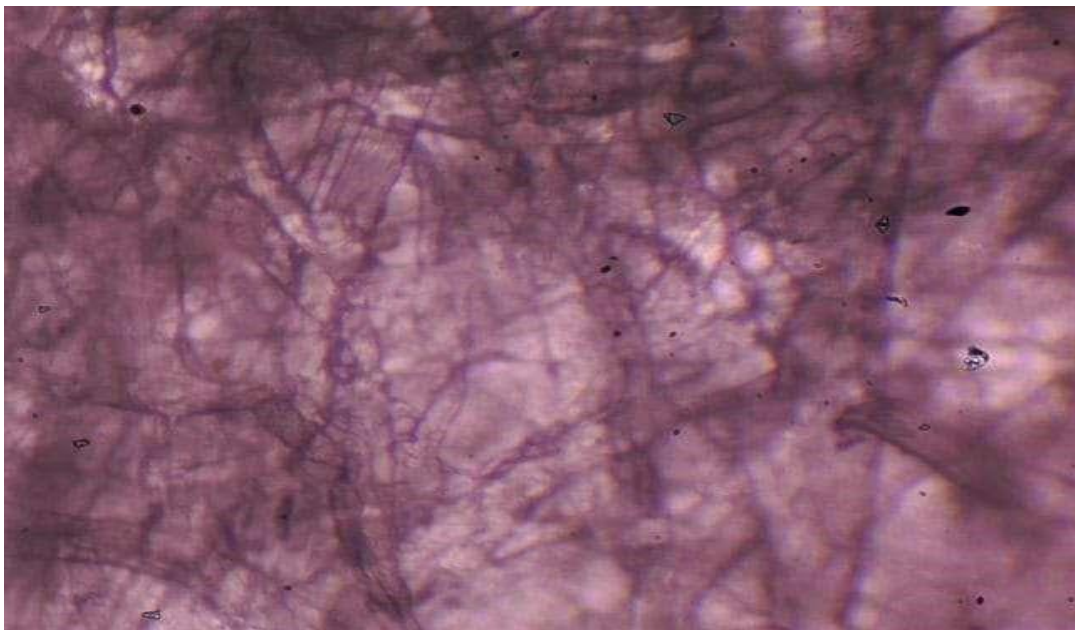


Fig. 2: Extraction and Characterization of Micro plastics

Statistical Examination

Appropriate statistical methods were used to assess the gathered data. Characteristic statistical parameters including mean and standard deviation (mean $\pm$ SD) were determined. For micro plastic abundance. The differences in microplastic concentration between the sampling locations were evaluated using a one-way ANOVA test. Fish consumption of microplastics was examined using Pearson's correlation analysis. Biometric characteristics. Including body length, body weight, and gut weight. At $p < 0.05$, statistical significance was taken into consideration.

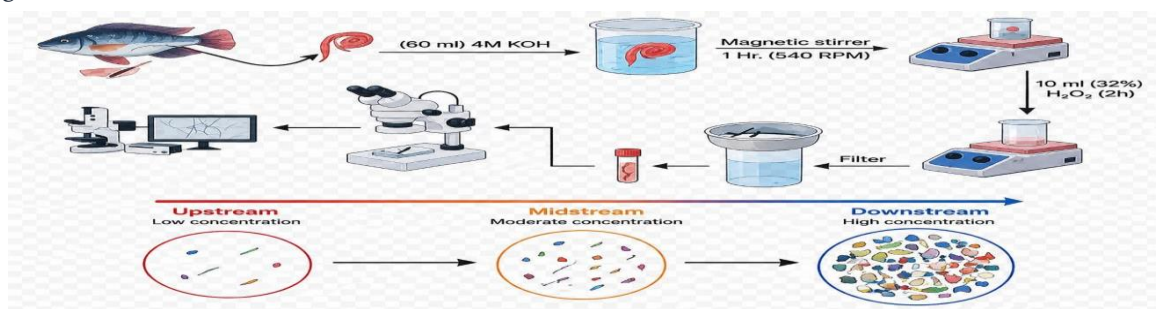


Fig. 3: Flowchart of Methodology

Results

Occurrence and Abundance of Micro-plastics

Out of the total 100 fish examined, 78% (n = 78) were found to contain micro plastics in their gastrointestinal tract. A total of 156 micro plastic particles were detected, with an overall mean abundance of **1.56 ± 1.21 items per fish**. These findings indicate a high prevalence of microplastic pollution in *Labeo rohita* obtained from the Indus River. The detailed summary of micro plastic occurrence is presented in **Tab. 1 & Fig.1**.

Table 1: Occurrence and Abundance of Micro-plastics in *Labeo rohita*

Parameter	Value
Total fish examined	100
Fish containing MPs (%)	78%
Total MPs detected	156
Mean MPs per fish	1.56 ± 1.21

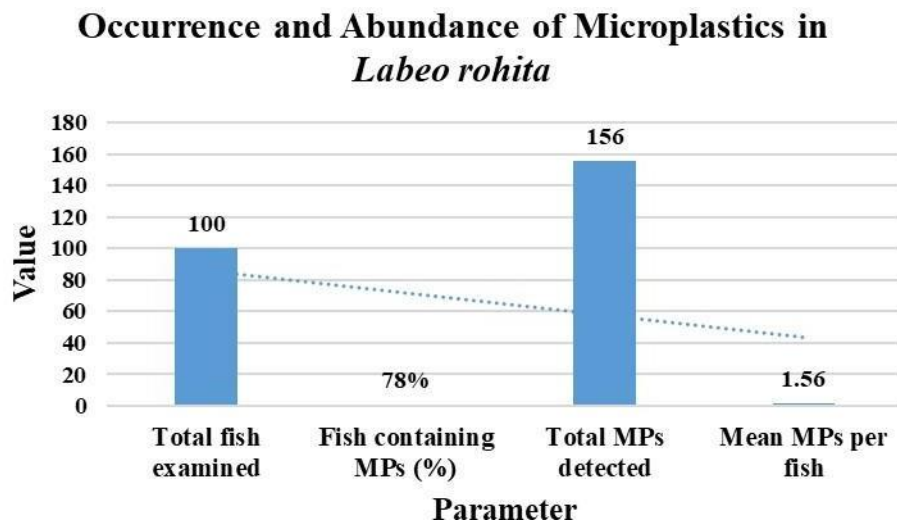


Figure 4: Occurrence and Abundance of Micro-plastics in *Labeo rohita*

Site-wise Distribution of Micro-plastics

Micro plastic abundance showed variation among the three sampling sites. The downstream site exhibited the highest mean micro plastic concentration (**2.05 ± 1.32 items/fish**),

followed by midstream (**1.51 ± 1.08 items/fish**) and upstream (**1.12 ± 0.95 items/fish**) sites. Similarly, the percentage occurrence increased progressively from upstream (68%) to downstream (88%), indicating increasing contamination along the river gradient.

Statistical analysis using a one-way ANOVA showed that the sampling sites differed significantly. ($F = 4.27$, $p = 0.017$; $p < 0.05$), confirming spatial variation in micro plastic contamination. These results are summarized in **Tab. 2 & Fig.5**

Table .2: Site-wise Distribution of Micro-plastics

Site-wise Distribution of Micro-plastics			
Site	No. of Fish	Mean MPs ($\pm$ SD)	% Occurrence
Upstream	34	1.12 ± 0.95	68%

Midstream	33	1.51 ± 1.08	79%
Downstream	33	2.05 ± 1.32	88%

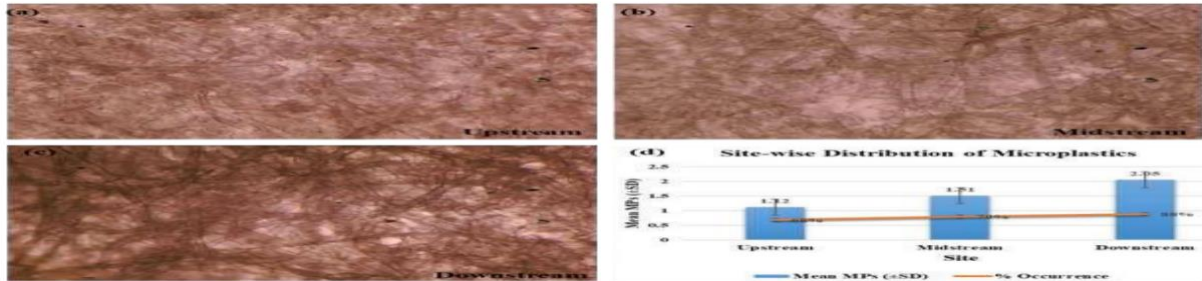


Figure 5: (a) Upstream Distribution of Micro plastics, (b) Midstream Distribution of Micro plastics
(c) Downstream Distribution of Micro plastics (d) Site-wise Distribution of Micro plastics.

Morphological Types of Micro-plastics

Micro plastics recovered from fish samples were categorized into five morphological types. With 67% of all particles, fibers were the most common type, followed by films (14%), lines (10%), fragments (6%), and foam (3%). The greater concentration of fibers particles indicate that the house hold waste water and textile dissolved fiber are main source of maps contamination in the present study region the distribution of MP's is morphologically summarized in **Tab 3 & Fig.6**.

Table 3: Morphological Types of Micro-plastics

Type	Morphological Types of Micro-plastics	
	Number	Percentage (%)
Fiber	104	67%
Film	22	14%
Line	16	10%
Fragment	10	6%
Foam	4	3%

Morphological Types of Microplastics

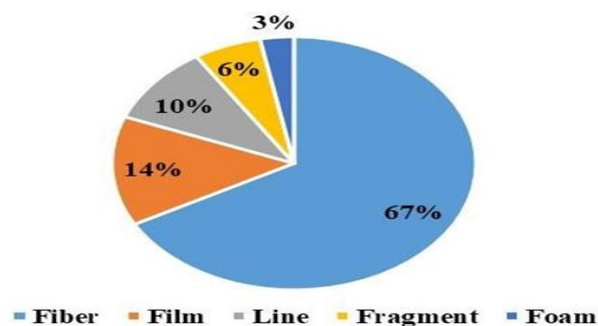


Figure 6: Morphological Types of Micro-plastics

Color Distribution of Micro-plastics

The extracted micro plastic particles show diverse color pattern. Suggestion the contamination of MPs from different pollution the black color particle of MPs were most abundant (29%) followed while green 3% this diversity of color variation reflect the different type of plastic material introduced into the fresh water ecosystem. The color wise distribution of MP's is show in **tab 4 & Fig.7**

Table 4: Color Distribution of Micro-plastics

Color	Percentage (%)
Black	29%
White	25%
Blue	22%
Red	16%
Green	8%

Color Distribution of Microplastics

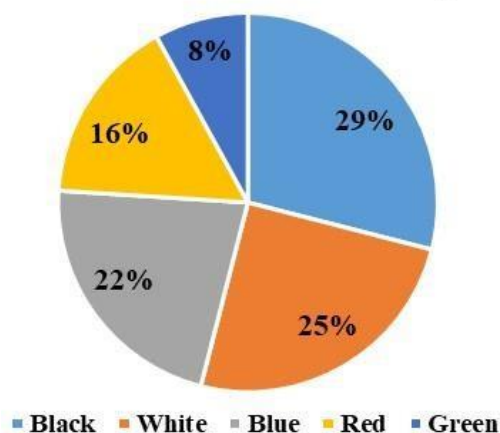


Figure 7: Color Distribution of Micro-plastics.

Correlation of MPs with Fish Biometric Characteristics.

The correlation analysis demonstrate the significant biometric relationship of fish with MPs ingestion. Body length ($R^2=0.41$, $p=0.021$) and body weight ($R^2=0.46$, $p=0.015$) are related to the number of ingested micro plastic, a significant relation was also observed between gut weight and MPs concentration. ($R^2=0.39$, $p=0.028$) over all these result indicate that the

larger fish have a greater tendency to ingest MPs particle's. The statistical result is given below in **tab. 5 & Fig.8.**

Table 5: Correlation between Micro-plastic Abundance and Fish Parameters

Parameter	Correlation between Micro-plastic Abundance and Fish	
	Parameters	
	R^2	p-value
Body length vs MPs	0.41	0.021
Body weight vs MPs	0.46	0.015

Gut weight vs MPs

0.39

0.028

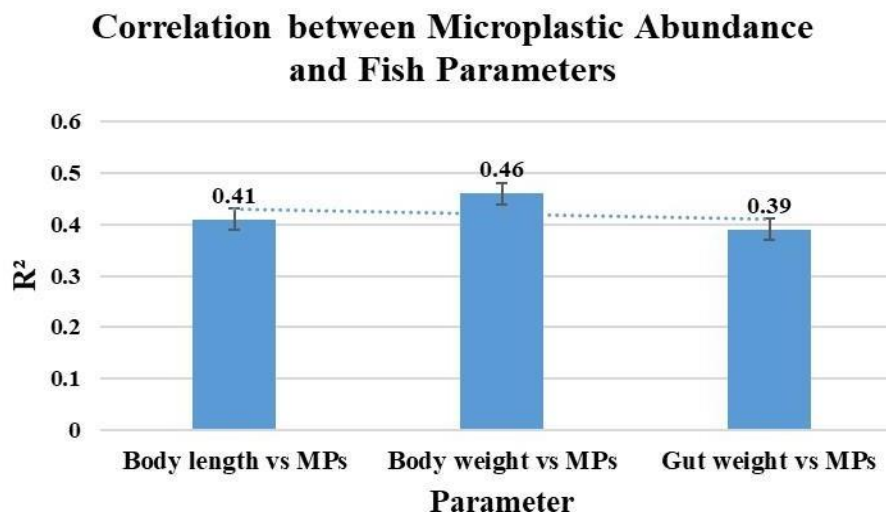


Figure 8: Correlation between Micro-plastic Abundance and Fish Parameters

Overall Statistical Significance

The present study overall statistical analysis show that MPs ingestion in *Labeo rohita* varied significantly among sampling site ($p < 0.05$) significant positive correlation with biometric parameter were also observed this result show that both environmental and biological condition lead to contamination of MPs in fish.

Discussion

The high incidence of present study revealed a high prevalence (78%) of MPs particles in freshwater fish *Labeo rohita* from Indus river Dera Ismail Khan. These findings indicate considerable MPs contaminating in fresh water ecosystem of D.I.Khan. This result is gently consistent with previous studies. Conducted on fresh water ecosystem worldwide although it is bit lower than those results recorded in other studies. For example, a study that found that 95% of fresh water fishes in China was contained with micro plastics (Jabeen et al., 2017), where 85% approximately prevalence was reported in Lake Michigan. (McNeish et al., 2018). Similarly, Sánchez et al. (2014) in addition the latest research confirmed the MPs ingestion in fresh water ecosystem of European rivers. The slightly low presentences was observed between the present study and previous reports also attributed with differences in local environmental pollution and waste management system. In this result the predominance of fiber shape particles were counted for 67% was observed in the present research investigation agree with other previous researches that have observed fiber as the most dominant MPs particles in fresh water ecosystem. This findings report support studies including those by Cesa et al. (2017) and Dris et al. (2015), which mark the synthetic fiber were most commonly originate from textile washing, and domestic sewage are the main source of fibers. Because many water management system cannot properly clean these particles, they eventually accumulate in fresh water. These findings have been documented in the Amazon and Yangtze River system (Pegado et al., 2018) That's why the predominance of fibers in the present study indicate that municipal wastewater and the degradation of fishing gear are major source of MPs pollution in Dera Ismail Khan. Additionally, The site wise investigation show a significant relation of microplastic abundance across three sampling site. This analysis show a higher concentration of MPs at downstream location of Indus River with p value ($P = 0.017$) with the highest abundance recorded at downstream sites. This research investigation is consistent with previous studies, showing highest downstream concentration of MPs because they receive continuous pollutants from upstream and midstream areas. Same observation is investigated by (Lebreton et al., 2017). The present study show that the higher abundance of MPs is associated with waste disposal, agricultural runoff and the plastic particles along the river bank. The micro plastic show variation in colors observed in this study show that the plastic particles come from different sources. Black (29%), White (25%), and Blue (22%) particles were mostly detected, followed by red and green. Similar color particles were detected in previous studies. These colors are directly linked to household waste material and textile fiber. For example, Bessa et al. (2018) investigate comparative analysis between marine and fresh water fish. He said that particle color provide useful information about MPs. The present study show that the MPs color show that the waste is come from domestic waste and industrial run off. In present study the significant relation between MPs and fish biometric parameter were found. These finding show that the MPs ingestion is depend on fish size including fish length, fish weight and fish gut. These finding show that large fish ingest more MPs as compare to

small individual. Similar investigation have done by Peters and Bratton and Jovanovic Who suggest that MPs contamination is linked with fish size and age. Large fish consume more MPs, more food and exposed to environment for long time period. The present study did not directly investigate trophic level, however previous finding show the effect of feeding habitat on MPs ingestion. While some researcher report the higher concentration in carnivores and omnivorous fish as a result of trophic transfer, (Nelms et al., 2018), other have observed no significant relation between diet and MPs ingestion (Pegado et al., 2018). The result of this study show that the fish and environment have a greater influence on MPs than feeding strategy. Therefore these finding indicate that environmental condition should also be considered when analyse the MPs ingestion in freshwater fish. Overall, the present research adds the scientific evidence that MPs possess a significant threat to freshwater fishes. The result is closely related to the previous studies confirming that MPs is a widespread environmental issue which is associated with human health. Addressing this issue, a proper waste management system is required, irregular monitoring are also needed to reduce MPs. Furthermore, additional research is needed on effect of MPs and long term ecological effect of MPs.

Conclusion & Mitigation Strategies

The present confirmed the MPs ingestion in freshwater fish *Labeo rohita* collected from Indus River, Dera Ismail Khan from three different site. Total 100 fish sample were collected, 34 from upstream, 33 from midstream and 33 from downstream. 78 fish sample show greater concentration of MPs in freshwater fish *Labeo rohita* with p value ($P = 0.017$). The fibers are most dominant particles about 67%, show black color. In this study we also observed a significant correlation with fish biometric parameter, it show that large fishes consume more MPs as compare to small fishes. These finding indicate that freshwater fish are more exposed to MPs pollution, posing potential ecological and human health concern. Effective waste management system, reduce MPs in open area, minimize the contamination of MPs in freshwater ecosystem.

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