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INVESTIGATES THE OCCURRENCE, TYPES, AND ABUNDANCE OF MICROPLASTICS ACCUMULATED IN FISH PLANILIZA ABU COLLECTED FROM HILLA RIVER, IRAQ

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ABSTRACT
Plastic pollution has quickly become one of the most enduring global environmental challenges, and microplastics (<5 mm) manifest as one of the most problematic fractions because they float through aquatic networks and infiltrate food webs. Fish can consume them either intentionally or inadvertently, resulting in the bioaccumulation of microplastics within their bodies and possibly transferring to higher trophic levels such as humans. Fish specimens (*Planiliza abu*) were obtained between December 2024 and June 2025 from 2 localities in Hilla River, central Iraq (during the wet and dry seasons) as a running aquatic system. 18 individuals returned per site and season were also captured. Three anatomical units were analyzed for MP accumulation: gills, digestive contents and digestive tissues. The samples were digested, stained with Nile Red and examined under a fluorescence microscope to detect microplastic particles. Microplastics of all shapes and sizes were found in all parts of fish samples. Spherical particles prevailed among contents of the digestive tract, whereas fragments, fibres and films were more abundant in the gills. Ankle morphologies were also observed in digestive tract contents. These results reveal the global distribution of microplastics in freshwater fish, increasing our awareness of their effects on humans through higher tropic levels.

KEYWORDS: Microplastics, Fish, *Planiliza abu*, Gills, Digestive tract, Hilla river

INTRODUCTION
Microplastics have been gradually recognized as a new type of global environmental emerging pollutions showing persistence, diversity and bioavailability across their divisions in the environmental matrix293031. They have multiple sources and can vary with size, shape, density, and to a lesser extent chemical composition(Rodríguez-Seijo& Pereira, 2017). Their small size makes them easily distribute in aquatic environments, where they might be inadvertently consumed by organisms. Consequently, microplastics have emerged as a serious threat to aquatic ecosystems and the health of the organisms living in them (Banaee et al., 2025).
Within living organisms, microplastics have the potential to induce effects such as oxidative stress and cellular damage, including impacts on normal body functions like growth and reproduction(Mao et al., 2022). Fish are especially sensitive because particles can be taken up directly via the gills in respiration, or indirectly through consumption while feeding(Ikuta et al., 2022).Microplastics can be integrated into higher-order species when they feed on contaminated lower-order species, enhancing the transference of such particles through the food web(Nelms et al., 2018). Differences in risks of exposures to microplastic among species that share the same system depend on habitat characteristics and feeding habits(De Sá et al., 2018).
The behaviour and fate of microplastics in the aqua-environment depend largely on the size of the particle. Ultra-fine particles, e.g., those below 150 µm, can penetrate the intestinal barrier and are carried to internal tissues and organs(Lusher et al., 2017). While the translocation is more probable when the particle size decreases, which may lead to fears of bioaccumulation and biomagnification. Furthermore, polymer type, particle shape and chemical compounds accompanying with microplastics may affect the potential risk to aquatic organisms(Rochman et al., 2019).
The most frequently reported fish tissues, which accumulate microplastics are gill and digestive organs (Harikrishnan et al., 2023). Their distribution in these organs indicates that both inhalation and oral ingestion are more significant exposure routes(Waheed et al., 2025). Gills especially represent one of the most important entry paths to fish tissues, since they have a large surface area with which water contact is continuous(Roda et al., 2020).Microplastics adhering to gills can reduce the effectiveness of respiration, leading to hypoxia and/or cause physical damage to gill filaments and making fish more susceptible for infection(Jabeen et al., 2018; Movahedinia et al., 2012) .Once ingested, microplastics can have an adverse effect in fish as a result of the physical obstruction and also pseudo-satiety involving chemical additives and co-carrying contaminants released into the gut(Strungaru et al., 2019).

It is therefore important to understand the presence of microplastics in freshwater fish as these contaminants also pose a risk for local ecology and may also provide a potential pathway for transfer into humans. The Hilla River which acts as the main tributary of the Euphrates has diversity of fish species and significant local economy, so it can be considered as a relevant system for investigating microplastic pollution in natural freshwater ecosystems.

MATERIALS AND METHODS

The Hilla River is a major branch of the Euphrates. It begins at Saddat al-Hindiyah and it flows southward with an average discharge around 200 m^3/s during summer and about 245 m^3/s in winter. Located in Babil Governorate, the river is about 101km long and serves as an important water source for both agriculture and local ecosystems. It supplies 36 first rank irrigation canals with a length of about 500 kilometers in Babil only, and increased the cultivated area in Babil, Qadisiyah and Muthanna Governorates .

For this study, two locations along the riverbank within the central urban area of Hilla City were selected as sampling sites. These sites represent different levels of anthropogenic influence: upstream before entering the city, and downstream after leaving the city (Figure 1).

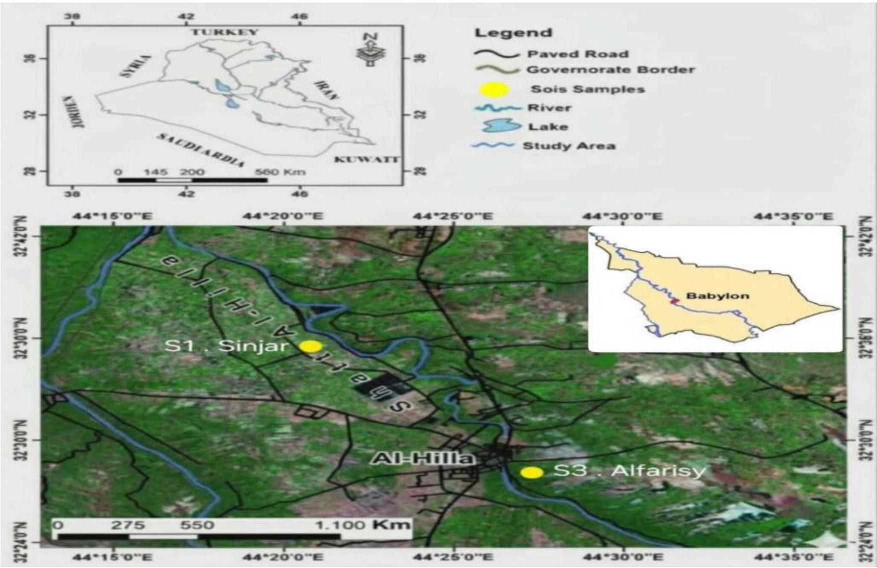


Figure (1) Map of study site on Hilla river .

Fish Sampling

Fish belonging to a single species (*Planiliza abu*) were collected from two stations, St.1 and St.2, during both the wet and dry seasons using traditional netting and catch methods. Immediately after collection, samples were placed in sterile containers and transported to the Advanced Environmental Laboratory at the Department of Biology, College of Science, University of Babylon. Species identification was confirmed upon arrival. This species was selected because it is abundant in the Iraqi waters, and constantly interacts with sediments and water column.(Figure 2).

Figure (2) Samples of Fish species (*Planiliza abu*) was used to extraction of MPs.

Extraction of Micro Plastics from Fish:

Isolation of microplastic was performed following the procedure in (Roch et al., 2020):

1. Sampling methods Each individual fish was weighed and measured for total length. The digestive system was carefully dissected, and the gills and content of digestive tract were removed individually for analysis.
2. Chemical digestion: Organic matter was digested (tissues of gills, digestive tract contents and digestive tract tissues) by placing each tissue type in a borosilicate beaker containing 25 from NaOH. Samples were then placed at 50°C for 15 min to digest biological materials.
3. The samples were diluted with 125 ml of distilled water and filtered by vacuum (via appropriate pore size filter paper) after digestion.
4. Microscopy: Retained material on the filter papers was examined under compound microscope at 40× magnification to observe the possible presence of microplastic particles.

Microplastic Identification

Post-extraction, microplastic-enriched residues were stained with the fluorescent dye Nile Red which is regularly used for plastic particle visualization. Attached to hydrophobic surfaces, the dye will produce fluorescence, indicating microplastic particles under a fluorescent microscope. For additional verification, the chemical composition of isolated particles and types of polymer were also identified by FTIR, by comparing the detected spectra to reference databases(Mao et al., 2022).

Statistical Analysis

All statistical analyses were conducted using JASP software (v17). Analyses of the data were performed by several methods:

- Analysis of Variance (ANOVA): to determine significant differences among sites, seasons, and tissue types.
- Boxplots: to illustrate variability and distribution patterns of microplastic concentrations.
- Principal Component Analysis (PCA): to visualize relationships among microplastic types and tissue locations.
- Two-way dendrogram clustering: to reveal similarity patterns among sampling sites and microplastic morphologies.
- Path diagrams: to explore potential cause–effect associations.
- Bar charts: to compare concentrations and morphological types across tissues.

These combined statistical techniques provided a comprehensive understanding of microplastic distribution and organ-specific accumulation patterns

RESULTS AND DISCUSSIONS

Microplastics were found in the gills, intestine and liver of *Planiliza abu* caught at both sampling sites along Hilla River which indicated contamination of those organs by MPs. The study was restricted to three tissues, i.e., gill, digestive content and the digestive tissues which each corresponds to a different pathway of exposure. Although organ tissues of the liver and muscles will be possible alternative tissues for microplastic exposure other than gills used in this study, it is known that gills and the digestive system are more sensitive compared to those of other internal organs, because they are continuously exposed to ambient water including its suspended particles (Abbasi et al., 2018). Results of this study further support such trend: the respiratory and feeding systems in fish contribute to the entry and retention of microplastics.

The digestive tissue in the gut also contained unambiguous indications of microplastic presence. This may be due to the selectivity permeability of the gut wall that only smaller particles can pass or are retained into tissue. Particles with sizes smaller than 5 µm can potentially penetrate the intestinal wall and be transferred to other tissues or organs (Liu et al., 2021). Such selective filtration may explain varied labor accumulations in different digestive parts.

The gills, on the other hand, showed an alternative pollution pattern. Their structure, flat filaments and broad surface area, renders them susceptible to microplastics suspended in water during breathing. Although the filtration capacity and inter-yarn distance contribute to why so considerable number of particles are caught by this region, environmental parameters also seem to play a role in the contamination (Amponsah et al., 2024). Our data indicate that different shapes of microplastics had different degrees of tissue distribution in the fish tested, with spheres, fragments, fibers and films being found in a descending order (Figure 3). This is consistent with previous findings in aquatic organisms which report corresponding dominant morphologies(Saikia&Handique, 2024).

Morphological differences are significant because the size and shape of microplastics often provide information on their origin (i.e. primary manufactured particles verses secondary fragments resulting from environmental decomposition). Those physical characteristics will also determine how microplastics are ingested by organisms and their level of physiological or ecological impact. In accordance with other studies, microplastics were recorded in gills and digestive tract of fish indicating the possibility of microplastics interference with fish health and fish behavior (Pan et al., 2021; Su et al., 2019).

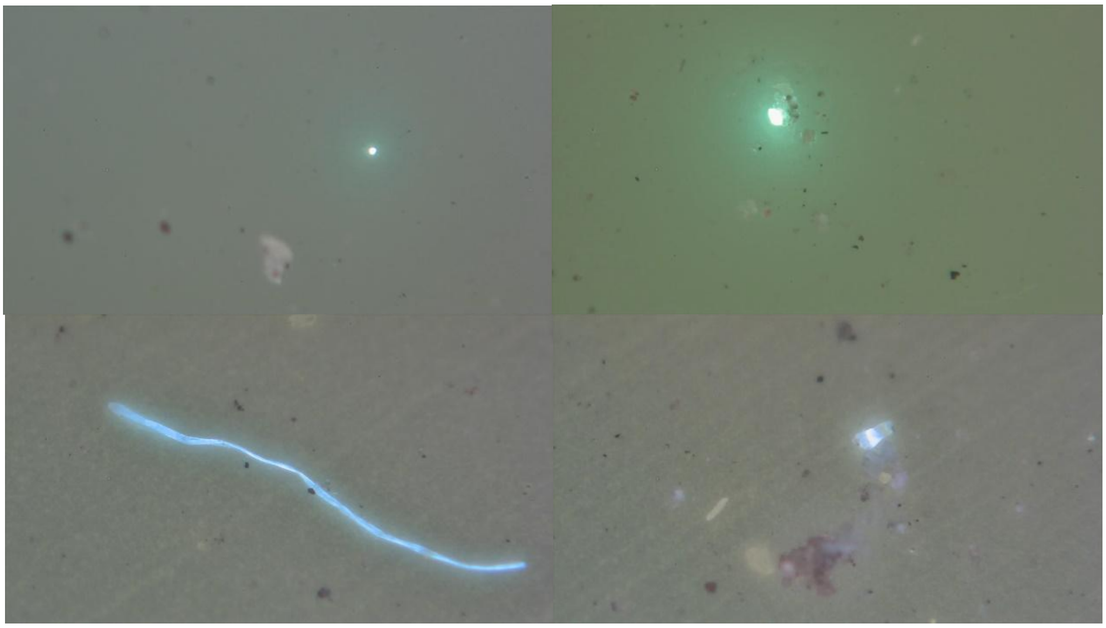


Figure (3) Fluorescent light microscopic images showing microplastic shapes in fish parts.

The concentration of spherical microplastics were marginally higher at station S3 10.53 (±0.891) and identical in the dry season with mean concentration of 10.240 (±0.775). The trend was also observed in the fragment particles, where during the wet season an average of 5.556 (±1.149) breaking events were recorded for S3. Fiber particles initially increased significantly at S3 also with levels of 0.777 (±0.271); dry season had almost the same at 0.778 (±0.270).

Film-type microplastics were overall low but increased slightly at S1 with an average concentration of 0.167 (±0.098) and, again, a small rise in the dry season at 0.204 (±0.102). Other morphological microplastics were also most prevalent in S3, with an average of 1.611 (±0.319) and during the dry season the values rose to be 1.926 (±0.347).

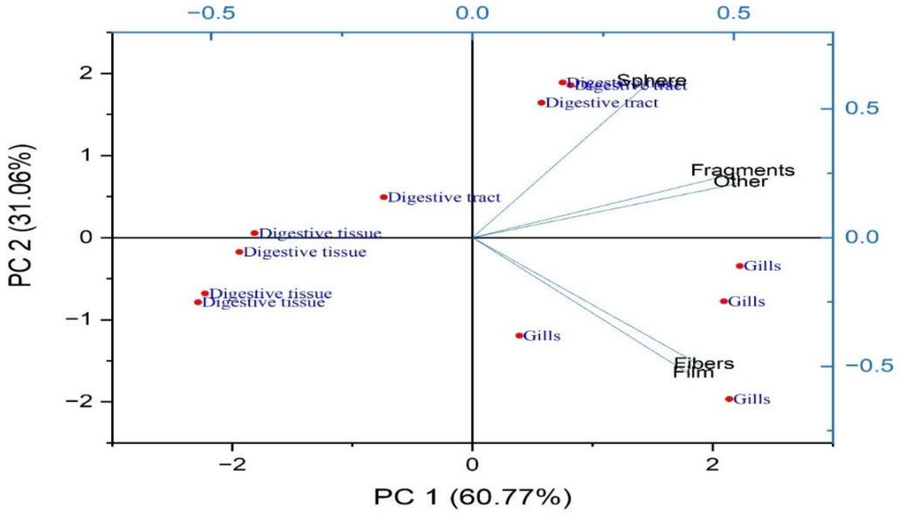
The high occurrence of spherical microplastics can be considered because they are present in personal care products and domestic refuse that may enter into freshwater via household effluent(Tibbetts et al., 2018). And, after being discharged into the water bodies, these large plastic materials are slowly disintegrated into tiny pieces that could either enter the food chain directly or indirectly damage aquatic life by releasing toxic chemical additives(Chen et al., 2020). Due to their size and shape, fragments are often confused with natural food sources which increases its chances of being ingested by fish (Saikia&Handique, 2024).

Microfibers are another major type of secondary microplastics, being derived primarily from the fragmentation of larger plastics by physical, chemical and biological mechanisms (Löder&Gerdts, 2015). There are two other primary sources of microfiber pollution,, which include breaking of fishing nets, ropes and gear, and fibers released by boating equipment and wastewater plants (Browne et al., 2011) .Among other microplastic types, fibers are more problematic in that they do not exit the gastrointestinal tractbut remain much longer; furthermore, they might accumulate to a greater extent in fish tissues (Hossain et al., 2023).

Figure (4) shows the results of principal component analysis depicting how types of microplastics are distributed in the fish tissues (gills, digestive tract contents and digestive tract tissue) that were screened. The plot indicates a positive correlation between the gills and fiber/film particles, and hence both of these forms are primarily retained in the gill systems.

On the other hand, digestive tract tissues position on the opposite side of PCA axis, reflect the specific microplastics profile in comparison to the other organs. The digestive tract contents, on the other hand, were more evenly distributed among spheres and fragments, as well as a series of unknown particle categories reflecting the range of materials traversing the gut during feeding.

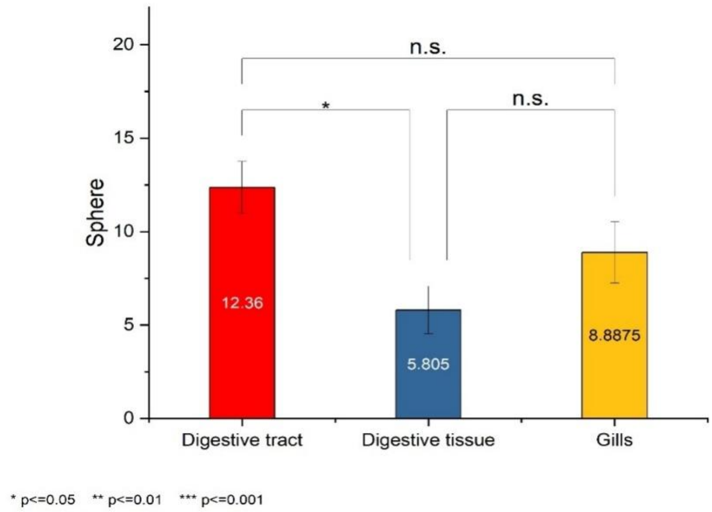
In general, the PCA identifies the two mechanisms via which microplastic enters the organism; respiratory activity seems to be most important for accumulation in the gills while feeding behavior contributes significantly towards retaining of microplastic within digestive tract (Pan et al., 2021)



Figure(4)Path Diagram of Principal Component Analysis(PCA)of MPs morphology in fish samples (gills, digestive tract contents, digestive tract tissues).

The highest concentrations of the spherical-sized particles were observed in the contents of the digestive tract, with an average concentration of 12.361 (±0.872). Following them, the gills 8.888 (±0.942), and the digestive tract tissues 5.804 (±0.688). Statistical comparisons found a difference between the digestive-tract content and tissues, but not between gills and tissues or gills and digestive-tract contents (Figure5).

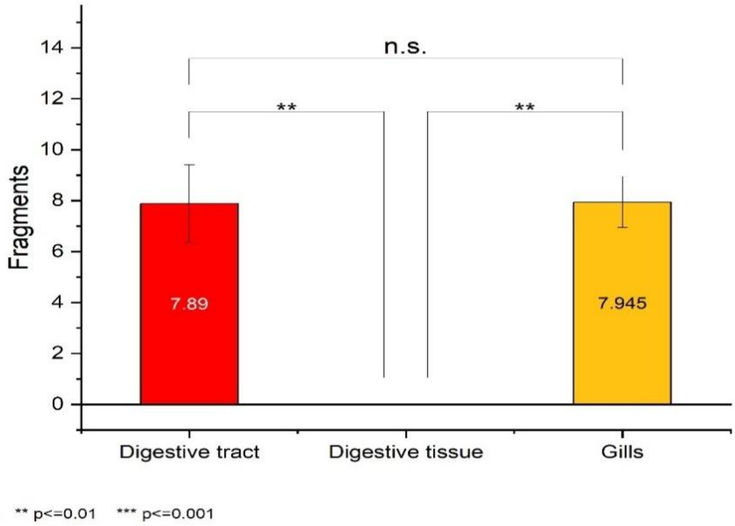
Higher microplastic scores in the digestive tract are predominantly attributed to ingestion directly, either via food uptake or through water and sediment intake. It could also be due to feeding on preys that themselves have taken up lower trophic level microplastics (Jovanović et al., 2018). An early study on microplastics in aquatic organisms also found round plastic particles in fish, clearly demonstrating the ease with which these particles may enter and remain in the digestive tract (Carpenter et al., 1972).



Figure(5) Bar chart of the distribution of MPs(sphere) in different parts of fish, including(gills, digestive tract contents, and digestive tract tissues).

The concentrations of microplastic fragments were highest in the gills (average 7.944 ± 0.569). The digestive contents came next to this with the average of 7.889 (±0.839). In contrast, there was no quantifiable fragment material detected in tissues of the gastrointestinal tract (all values were 0.00 (0.00)). Statistical analysis demonstrated that the gills and digestive tract tissues, as well as the digestive tract tissues and digestive tract contents differed significantly, but not between the gills and digestive tract contents (Figure 6).

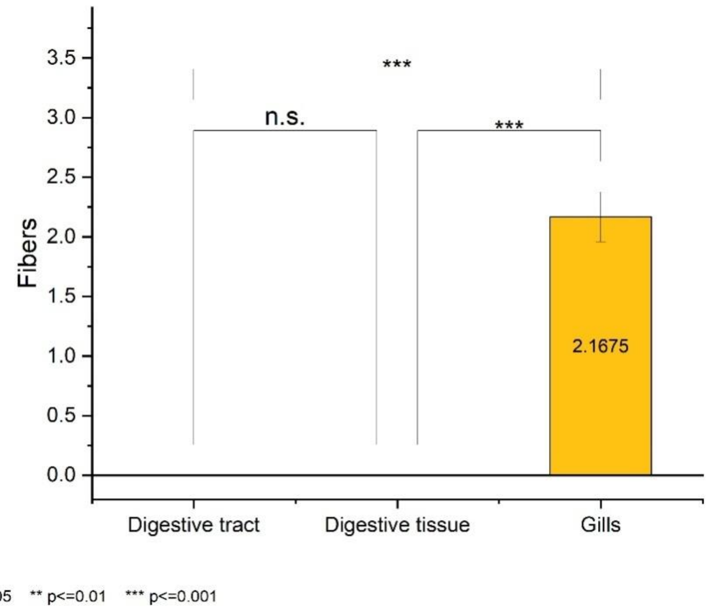
The fragment particles normally have a rough or worn surface, the shape being rounded and with smoothed edges, angular and irregular with visible slits(Tanaka & Takada, 2016). Mostly they are regarded as passively involved, through breathing, in the gill. During aeration, water passes through the gill chambers and it is likely that suspended particles such as microplastics are incidentally accumulated by (and remain in) gills as a result of their sticky surface structure(Zhang et al., 2021). Results such as these have also been described by other authors, in which fragments are commonly found in the gills of exposed fish (Rasta et al., 2023).



Figure(6) Bar chart of the distribution of MPs(fragments) in different parts of fish, including(gills, digestive tract contents, and digestive tract tissues).

The highest number of microplastic fibers were observed in the gills and a maximum concentration of 2.1675 (±0.139) was obtained. However, the digestive tract tissues and digestive tract contents displayed undetectable levels of fiber particles with concentration of 0.00 (±0.00). Statistical analysis confirmed a significant difference between the gills and each of the digestive tract components, while no significant difference was found between the digestive tract tissues and digestive tract contents (Figure 7).

The tendency for fiber particles to accumulate in the gills may be linked to the anatomical structure of the gill filaments. Their comb-like arrangement allows them to retain suspended fibers more efficiently as water passes over them during respiration(Pan et al., 2021). Similar findings have been reported in previous studies, which likewise observed a higher abundance of microplastic fibers in the gills compared with other organs (Erdoğan, 2025; Pan et al., 2021; Rasta et al., 2023).



Figure(7.) Bar chart of the distribution of MPs(fibers) in different parts of fish, including(gills, digestive tract contents, and digestive tract tissues).

Here most concentrated in the gills, with an average of 0.473 (±0.133). In contrast, both the digestive tract contents and digestive tract tissues recorded no detectable film particles, remaining at 0.00 (±0.00). Statistical analysis revealed significant differences between the gills and each of the digestive components, while no significant difference was found between the digestive tract contents and tissues(Figure 8).

Previous studies have similarly reported the presence of film particles in fish gills (Irnidayanti et al., 2023; Rasta et al., 2023).The current findings also demonstrate that the gills contain higher amounts of fibers, films, and fragments compared with other organs. This pattern is consistent with the idea that microplastic accumulation in the gill structures often occurs incidentally and without selective uptake, as water continuously flows across the gill filaments during normal respiration(Su et al., 2019).

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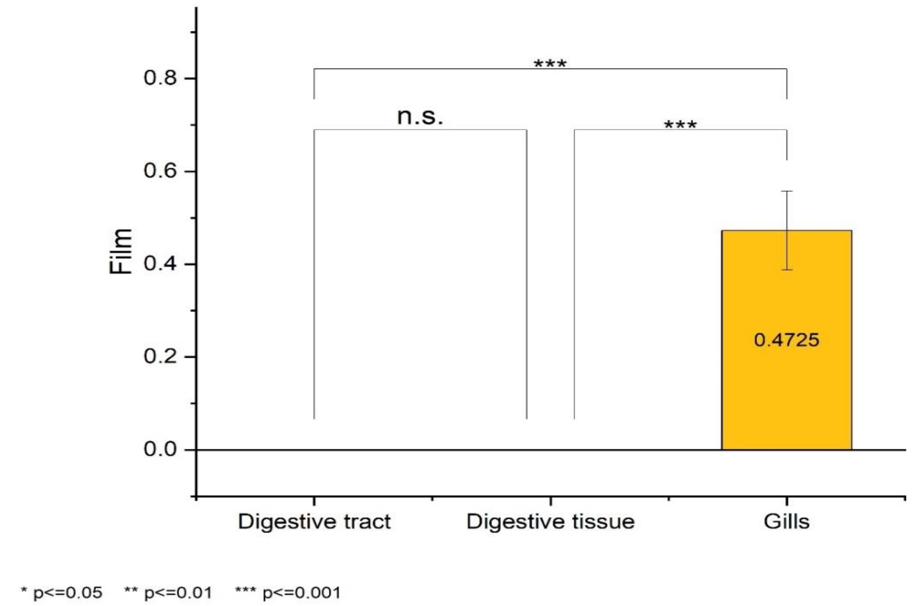
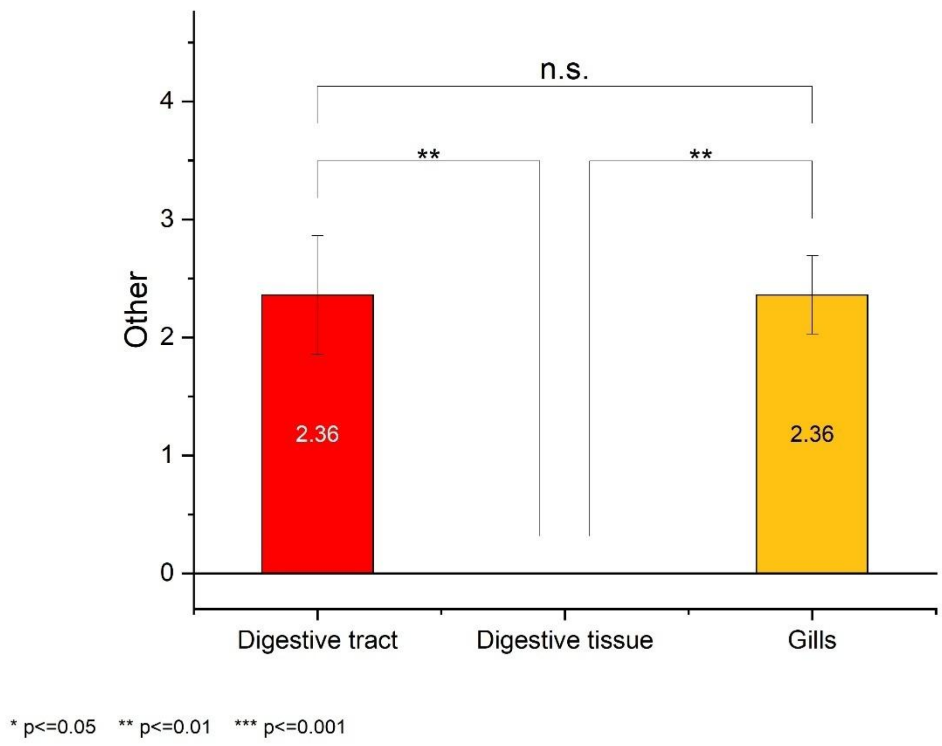


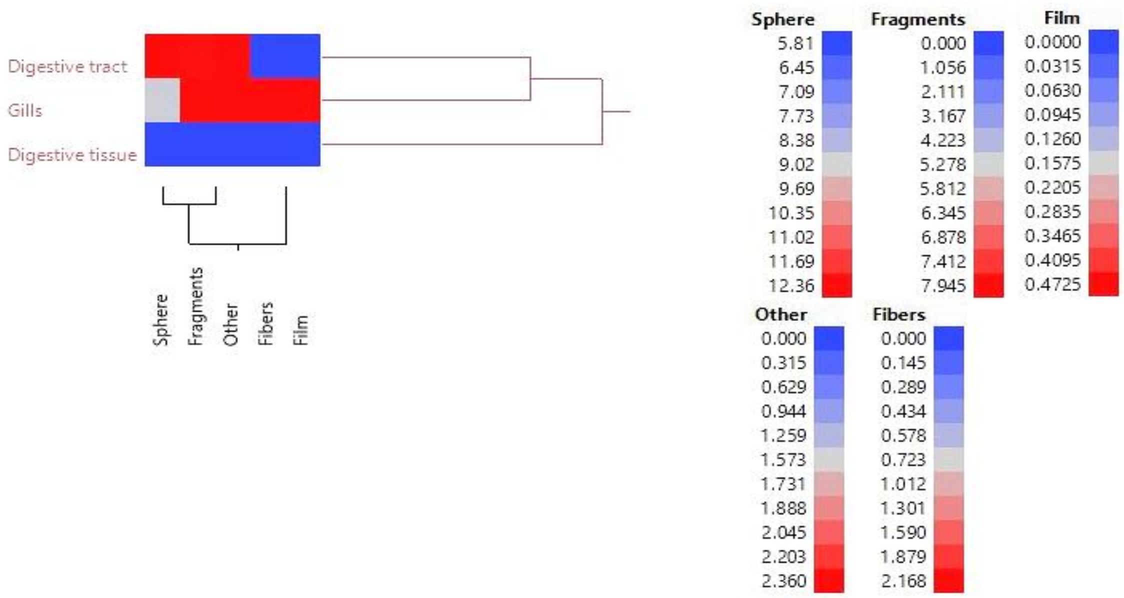
Figure(8) Bar chart of the distribution of MPs(film) in different parts of fish, including(gills, digestive tract contents, and digestive tract tissues).

The results also showed the presence of other microplastic morphologies. Their highest concentration appeared in the digestive tract contents, with an average of 2.362 (±0.289). This was closely followed by gills Average 2.360 (±0.199). The particles of this nature were not detected in the tissues of the digestive tract, and remained localized as 0.00 (±0.00) (Figure9). Statistical analysis showed a significant difference between the gills and tract tissues, and tract contents from the same fish. However, there was no significant difference between the gills and digestive tract contents.



Figure(9) Bar chart of the distribution of other morphology of MPs in different parts of fish, including(gills, digestive tract contents, and digestive tract tissues).

The dendrogram indicates the levels of commonality and dissimilarity between fish organs (gills, digestive tract contents, and digestive tract tissues) against the different morphological shape of microplastics. Cluster 1 was characterized by the lowest similarity of digestive tract contents and tissues indicating distinct differences in their microplastic characteristics. Instead, Cluster 2 exhibits the greatest similarity between gills and digestive tract content, indicating that these two organs might exhibit a similar distribution pattern on microplastic load.
In terms of the particle morphologies, they are clustered as follows: Cluster 4 indicates that film and fiber particles are most like each other, followed by Cluster 1, in which spherical and fiber particles share the least similarity. These relationships are based on the concept that similar drawing is smaller when distance between clusters is longer and vice versa (Figure 10).



Clustering History

Number of Clusters	Distance	Leader	Joiner
2	1.886975725	Digestive tract	Gills
1	2.537582041	Digestive tract	Digestive tissue

Clustering History for Columns

Number of Clusters	Distance	Leader	Joiner
4	1.570092e-16	Fibers	Film
3	0.006015883	Fragments	Other
2	0.639261635	Sphere	Fragments
1	1.755884446	Sphere	Fibers

Figure(10) Dendrogram Two Way MPs morphology in fish.

CONCLUSIONS

This research effectively quantified and identified microplastic particles in the gills, intestinal contents, and intestinal tissues of *Planiliza abu* taken from Hilla River. The data indicate that this species is susceptible to microplastic pollution, with spheres and fibers being the most common morphotypes in different proportions on the studied tissues confirmed by be presence of these two types. There were significant differences between the gills and both types of digestive tract, but not between digestive tract contents and tissues.
In addition, the gills also contained higher levels of fibers, films and fragments than other tissues. Such a pattern is attributed to the passive and non-selective nature of microplastic uptake in the gill, as particles within water flow are able to be entrained passively during respiration not selectively taken up, attaching readily on the fragile gills due to their sticky surface properties. This involuntary exposure is why the gills are frequently a major site of entry for microplastics. While the present study has unveiled valuable information about the occurrence and distribution of microplastics in freshwater fish, more work is required to fully comprehend the physiological effects of these particles on *Planiliza abu* as well as other species living in the river. Further research is needed

to study possible human health hazards in humans, especially in areas where eating fish is an important part of the diet.

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Conflict of Interest
No Conflict of Interest.
Author Contributions
T. Hyder conducted the experiments, collected the data, and performed the analyses. J.M. Salman conceived the study idea, designed its objectives and editing the manuscript. M.J. Yase contributed to writing, reviewing, and editing the manuscript, as well as discussing the results.

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