Evidence of microplastic contamination in the food chain: an assessment of their presence in the gastrointestinal tract of native fish

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Abstract

This research study focused on the primary freshwater surface sources of Khyber Pakhtunkhwa (KP) province, Pakistan—Swat and Kabul rivers. The study aimed to assess microplastic (MP) contamination of fish in the Swat and Kabul rivers. Understanding this contamination is vital for evaluating the environmental and health risks associated with consuming contaminated fish and contributing to the ongoing conservation efforts. The study’s objective was to investigate the presence of MPs in the gastrointestinal tracts (GIT) of fish and to delineate the identified MP types. Samples of local dominant fish (Schizothorax plagiostomus [Swatay] and Racoma labiata [Chunr], Cyprinus carpio [Common Carp], and Clupisoma naziri [Sher Mayai]) were collected, and their body weight and length assessed to gauge the overall health. The GIT samples were processed, digested and filtered before microscopic examination to detect MPs. Subsequently, the identified MPs were subjected to attenuated total reflectance–Fourier transform infrared spectroscopy for characterization by analyzing their absorption bands. Results of the study showed the presence of MPs in fish samples, predominantly identified as polyethylene (PE), with polypropylene (PP) being the subsequently prevalent plastic type, which manifested fish contamination with MPs. The study revealed MP pollution in both Swat and Kabul rivers, with fish ingesting these particles. This poses potential health risks for fish and health of the ecosystem.

Keywords: bioaccumulation; ecosystem health; ingestion; pollution; rivers Swat and Kabul

Introduction

Globally, the production of plastic is increasing rapidly due to its low production cost. In 2018, the total production of plastics reached 359 million tons (Plastics Europe, 2019). While plastic brings tremendous comfort to our daily lives, its high production and usage rates have accumulated vast amounts of plastic waste. It is estimated that around 4,900 million tons of plastic waste has accumulated in the environment, and by the
Materials and Methods

Study area

The study focused on the primary freshwater surface water sources of Khyber Pakhtunkhwa (KP) province in Pakistan, specifically Swat and Kabul rivers. In all, 61 fish samples were collected from these rivers. Fish samples were collected from different locations, selected based on major waste drain entry points, mixing zones, and potential fish capture sites at Swat and Kabul rivers. Upstream locations were chosen, with Madyan/Behrain on Swat river and Warsak Dam on Kabul river identified as upstream sites. In total, samples were collected from 10 locations mentioned in Table 1 at both rivers, namely Madyan/Behrain (MS), Khwaza Khela Swat (KKS), Barikut Swat (BS), Landakay Swat (LS), Takhtaband/SherAbad Swat (TS), Chakadara bridge (CS), Warsak Dam (WK), Charsadda bridge (CK), Pir Sabaq (PK), and Khair Abad (KS). The map along with GPS locations of the sampling sites are shown in Figure 1.

Fish samplings and preservation

At each sampling site, we collected native fish species demonstrating local dominance. The following fish species were collected from Swat river: Schizothorax plagiostomus (commonly known as Swatay) and Racoma labiata (referred to as Chunr). The prevalent native species in Kabul river comprised Cyprinus carpio (commonly known as Common Carp) and Clupisoma naziri (also known as Sher Mayai). The collection of these dominant native fish species from both Swat and Kabul rivers involved the use of fishing rods, fishhooks, and nets.

Within 1 h of capture, the fish samples were wrapped in aluminum foils and stored at low temperatures. To ensure proper identification and record-keeping, zip-lock polyethylene bags were utilized to encase fish samples, with labels indicating the sampling location, date, and specific species. The specimens were promptly dissected on the day of collection to excise carefully the GIT. Subsequently, materials in the gut were meticulously preserved in containers containing a prepared solution of Potassium Hydroxide (KOH), optimized for digestion purposes (Wang et al., 2021). Deionized water was used for cleaning instruments and preparing KOH solution. Additionally, all solutions, including the KOH solution, were filtered through glass microfiber filters to prevent external contamination.

Length and weight measurements

Following collection, the fish were weighed to the nearest 0.01 g using a digital balance and measured for total length and weight. The fish specimens were promptly dissected on the day of collection to excise carefully the GIT. Materials in the gut were meticulously preserved in containers containing a prepared solution of Potassium Hydroxide (KOH), optimized for digestion purposes (Wang et al., 2021). Deionized water was used for cleaning instruments and preparing KOH solution. Additionally, all solutions, including the KOH solution, were filtered through glass microfiber filters to prevent external contamination.

Evidence of microplastic contamination in the food chain

Microplastics are present in different parts of the environment, such as air, water, and soil (World Health Organization [WHO], 2019; Yu et al., 2024; Abassi et al., 2024; Naveed et al., 2023a; Naveed et al., 2023b; Zameer et al., 2023). The substantial quantity of MPs poses an ecological risk, leading to food contamination and affecting human health (Barboza et al., 2018). In 2014, marine ecologist Richard Thompson reported the distribution of MPs in oceans for the first time (Law and Thompson, 2014). Several studies have also reported the presence of MPs in commercially caught marine fish species (Bellas et al., 2016; Rochman et al., 2015), indicating high exposure to MPs contamination in fish populations. In the North Pacific Central Gyres, a study confirmed the presence of MPs in the gut samples of planktivorous fish (Boerger et al., 2010). Furthermore, MPs have recently been detected in human blood samples, entering the body via contaminated food or water (Leslie et al., 2022). Recent studies have focused on the occurrence and distribution of MPs in freshwater and terrestrial ecosystems (Rochman, 2018). Studies on freshwater fish contamination have shown the presence of MPs in the gastrointestinal tracts (GIT) of fish, confirming their presence in freshwater (Sanchez et al., 2014). Globally, MPs have developed a great interest because they enter into food chains and potential health risks, such as toxicity (Khan et al., 2023; Akram et al., 2023; Xiong et al., 2022). This study aimed to assess the occurrence, distribution, types, and abundance of MPs in the GIT of fish in both Swat and Kabul rivers. Fish is collected for locally consumption from both rivers; however, fishing is mainly carried out for recreational purpose and a very small amount for commercial purposes. Sampling locations were selected under waste ingress locations and potential fish capture sites.

It was also estimated that 12.2 million tons of plastic enter the ocean annually (Jambeck et al., 2015) through various pathways. Large-size plastic waste that poses an ecological problem breaks down into smaller particles over time (Napper and Thompson, 2019). Although no plastics biodegrade significantly, environmental factors, such as sunlight, weaken the materials, causing them to break into fragments of millimeter to micrometer in size (Andrady, 2015). Microplastics (MPs) are found in abundance among these small-sized particles (Arthur et al., 2009). MPs originate from primary and secondary sources. Primary MPs are produced industrially for specific domestic and industrial uses, such as cosmetics and the plastic industry, while secondary MPs result from the disintegration of large plastics because of biological and physiochemical breakdown. MPs undergo various physiochemical and biological weathering processes in the environment (Jahnke et al., 2017).

Microplastics are present in different parts of the environment, such as air, water, and soil (World Health Organization [WHO], 2019; Yu et al., 2024; Abassi et al., 2024; Naveed et al., 2023a; Naveed et al., 2023b; Zameer et al., 2023). The substantial quantity of MPs poses an ecological risk, leading to food contamination and affecting human health (Barboza et al., 2018). In 2014, marine ecologist Richard Thompson reported the distribution of MPs in oceans for the first time (Law and Thompson, 2014). Several studies have also reported the presence of MPs in commercially caught marine fish species (Bellas et al., 2016; Rochman et al., 2015), indicating high exposure to MPs contamination in fish populations. In the North Pacific Central Gyres, a study confirmed the presence of MPs in the gut samples of planktivorous fish (Boerger et al., 2010). Furthermore, MPs have recently been detected in human blood samples, entering the body via contaminated food or water (Leslie et al., 2022). Recent studies have focused on the occurrence and distribution of MPs in freshwater and terrestrial ecosystems (Rochman, 2018). Studies on freshwater fish contamination have shown the presence of MPs in the gastrointestinal tracts (GIT) of fish, confirming their presence in freshwater (Sanchez et al., 2014). Globally, MPs have developed a great interest because they entered into food chains and potential health risks, such as toxicity (Khan et al., 2023; Akram et al., 2023; Xiong et al., 2022). This study aimed to assess the occurrence, distribution, types, and abundance of MPs in the GIT of fish in both Swat and Kabul rivers. Fish is collected for locally consumption from both rivers; however, fishing is mainly carried out for recreational purpose and a very small amount for commercial purposes. Sampling locations were selected under waste ingress locations and potential fish capture sites.
Table 1. Sampling locations on Swat and Kabul rivers.

<table>
<thead>
<tr>
<th>Location</th>
<th>Abbreviation</th>
<th>GPS coordinates</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Swat river</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Bahrain Swat</td>
<td>MS</td>
<td>N 72.57335 E 35.23086</td>
<td>2</td>
</tr>
<tr>
<td>2. Khwaza Khela Swat</td>
<td>KKS</td>
<td>N 72.45113 E 34.94386</td>
<td>1</td>
</tr>
<tr>
<td>3. Barikot Swat</td>
<td>BS</td>
<td>N 72.30893 E 34.78667</td>
<td>12</td>
</tr>
<tr>
<td>4. Landakay (Shamazo bridge)</td>
<td>LS</td>
<td>N 72.21085 E 34.68334</td>
<td>25</td>
</tr>
<tr>
<td>5. (Sher Abad) Takhtaband Swat</td>
<td>TS</td>
<td>N 72.12792 E 34.66443</td>
<td>5</td>
</tr>
<tr>
<td>6. Chakdara bridge</td>
<td>CS</td>
<td>N 72.02912 E 34.64140</td>
<td>5</td>
</tr>
<tr>
<td><strong>Kabul river</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Warsak Kabul</td>
<td>WK</td>
<td>N 71.40807 E 34.17423</td>
<td>2</td>
</tr>
<tr>
<td>8. Charsadda moterway bridge</td>
<td>CK</td>
<td>N 71.73481 E 34.09274</td>
<td>3</td>
</tr>
<tr>
<td>9. Pir Sabaq</td>
<td>PK</td>
<td>N 72.04987 E 34.01117</td>
<td>4</td>
</tr>
<tr>
<td>10. Khairabad bridge</td>
<td>KS</td>
<td>N 72.21676 E 33.965344</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>61</td>
</tr>
</tbody>
</table>

The sampling took place in distinct rounds in July 2022 and February 2023. Fish were caught using nets, and detailed data concerning their species, weight, and length were meticulously recorded after being caught using balance and a ruler.

length to the nearest 0.01 cm using a measuring tape. Fulton’s condition factor (K) was calculated using the following equation:

$$K = \frac{\text{Total body weight (g)}}{\text{Total length (mm)}^3}$$

**Dissection**

Fish samples were dissected using a razor blade or a pair of sharp scissors, along with forceps, ensuring that all tools were washed with deionized water and air-dried to prevent any contamination. The dissection of fish samples to collect the GIT followed a methodology described in previous studies (Lusher et al., 2013; Xu et al., 2024a). Contents of the GIT were collected in a glass jar/container.

**Digestion**

In order to isolate MPs from the GIT, it is essential to digest organic matter. However, the use of strong oxidizing agents, particularly at increased temperatures, can potentially damage or degrade synthetic polymers (Welden and Lusher, 2017). Recent studies on GIT digestion have suggested that KOH is the most suitable agent for this purpose (Dehaut et al., 2016; Xu et al., 2024b).

The GITs were transferred to glass jars or beakers, with 10% KOH solution. The volume of KOH solution added to the jars was determined based on the volume of GIT. Subsequently, these solutions containing biological materials were incubated at 60°F for 5 days to ensure complete digestion. A blank check was performed concurrently with other samples to monitor for any potential contamination. The specimens were stored in a dark laboratory environment and consistently agitated to ensure thorough digestion.

**Filtration**

After the 5-day digestion period, the solutions underwent filtration using a vacuum filtration unit equipped with 0.47-μm microfiber filters. The resulting filter papers were then dried in an oven at 60°C and securely stored in petri dishes to prevent any potential external contamination.
Microscopic inspection, identification, characterization, and Fourier transform infrared (FTIR) analysis

Glass microfiber filters enclosed in petri dishes were visually observed with the naked eye. Subsequently, a stereomicroscope (CX41; Olympus Co. Ltd., Japan) coupled to a digital camera (Nikon DS Ri2) was employed to examine the filters containing recovered MP particles. The examination was conducted at total magnifications ranging from 4× (minimum) to 10× (maximum) to identify, quantify, and measure MPs using a ruler. Concentrations of MPs were expressed as the number of particles per sample. Each sample was analyzed individually, and four major categories of MPs based on morphology (foam, fibers, fragments, and pellets) were identified (Hidalgo et al., 2012). During microscopic analysis, the count of MPs present on each filter paper was documented. Additionally, the color and size of each potentially identified MP was recorded, following the approach outlined in the study conducted by Su et al. (2016).

Attenuated total reflectance (ATR)–FTIR spectroscopy was employed for the characterization of MP particles. A Perkin Elmer Spectrum 65 FTIR spectrometer with a LiTa detector and a universal attenuated total reflectance (UATR) ZnSe crystal with a 3–4-mm aperture were used to capture spectra in the range of 4,000–5,000 cm⁻¹.

Statistical analysis

For statistical analysis, a simple t-test was employed to compare different results. Additionally, maximum, mean, and standard deviation values of MP samples were calculated.

Results and Discussions

Fulton’s condition factor

The body weight and length ratio were calculated separately for each sample of fish species collected during sampling. The calculated K factor values for different species are provided in Table 2.
Table 2. Fulton’s condition factor (K) calculated for collected samples of individual fish species.

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Common name</th>
<th>No. of fish samples</th>
<th>Body weight (gm)</th>
<th>Standard deviation for weight</th>
<th>Length (cm)</th>
<th>Standard deviation for length</th>
<th>Fulton’s condition factor (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schizothorax plagiostomus</td>
<td>Swatay</td>
<td>29</td>
<td>72.95</td>
<td>44.26</td>
<td>19.19</td>
<td>3.94</td>
<td>1.27</td>
</tr>
<tr>
<td>Cyprinus carpio</td>
<td>China kub/paplait</td>
<td>12</td>
<td>55.89</td>
<td>24.12</td>
<td>14.23</td>
<td>2.58</td>
<td>1.31</td>
</tr>
<tr>
<td>Racoma labiatus</td>
<td>Chunr</td>
<td>15</td>
<td>38.20</td>
<td>8.66</td>
<td>14.41</td>
<td>0.86</td>
<td>0.88</td>
</tr>
<tr>
<td>Clupisoma naziri</td>
<td>Sher Mayai</td>
<td>05</td>
<td>79.02</td>
<td>21.21</td>
<td>23.05</td>
<td>1.48</td>
<td>1.14</td>
</tr>
</tbody>
</table>

The results revealed mean K values for S. plagiostomus, C. naziri, and C. carpio to be higher than 1, indicating good health of these species in their habitat. Conversely, Racoma Labiatus showed K < 1, suggesting poor health of this species in its habitat, possibly because of various factors, such as insufficient food supply, ecological stress, modifications in physicochemical parameters, and/or pollution (Akhtar et al., 2021; Zhang et al., 2023). According to Hussain et al. (2009), the heavier the fish with respect to its length, the higher its K value. Higher K values (≥1) indicate good conditions for both fish and its habitat, signifying adequate availability of feed.

Microscopic observations

Microscopic analysis through a stereo microscope identified 67 MP particles with different morphologies, such as pellets, fibers, and fragments. In all, 33 fish samples with MPs were identified (details provided in Table 3). Concerning size, only seven MPs had a size > 0.1 mm, while all the remaining MPs had a size ≤ 0.1 mm (11 MPs > 0.1 mm, and 56 MPs ≤ 0.1 mm). The average number of MP particles per fish sample was 1.10 with a standard deviation of 12.98.

In all, 50 fish samples were caught from six locations of river Swat, and 59 MPs were identified in these samples. In all, 11 fish samples were caught from four locations of river Kabul, with eight MPs identified. Location wise on both rivers, 25 fish samples were collected from Landakay Swat, with 16 samples revealing the presence of 43 MPs. Comparatively, the upper areas of river Swat, specifically the Bahrain samples, showed no identified MPs and therefore designated as the reference point. The number of fish samples and MPs discovered in the samples are illustrated in Figure 2.

Stereomicroscopic evaluation of MP particles concerning color was carried out; predominant colors identified were green, blue, yellow, and transparent colors (Saeed et al., 2022). Shown in Figure 3 are different colors and shapes of microplastics identified under the microscope.

Size analysis indicated that size of 16.42% (11 particles) of MPs ranged from 100 µm to 470 µm, and the remaining

Table 3. Microplastic abundance in fish samples collected at each location and number of microplastics per location.

<table>
<thead>
<tr>
<th>Location</th>
<th>Abbreviation</th>
<th>No. of samples</th>
<th>Samples with MPs</th>
<th>No. of MPs</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Swat river</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Madyan/Bahrain Swat</td>
<td>MS</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2. Khwaza Khela Swat</td>
<td>KKS</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3. Barikot Swat</td>
<td>BS</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>4. Landakay (Shamazo bridge)</td>
<td>LS</td>
<td>25</td>
<td>16</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>5. (Sher Abad) Takhtaband Swat</td>
<td>TS</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>6. Chakdara bridge</td>
<td>CS</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td></td>
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<tr>
<td><strong>Kabul river</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Warsak Kabul</td>
<td>WK</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8.Charsadda moterway bridge</td>
<td>CK</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>9. Pir Sabaq</td>
<td>PK</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>10. Khairabad bridge</td>
<td>KS</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>61</td>
<td>33</td>
<td>67</td>
<td>12.98</td>
</tr>
</tbody>
</table>
Evidence of microplastic contamination in the food chain

<table>
<thead>
<tr>
<th>No. of fish samples</th>
<th>No. of fish samples with MPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

KKS BS LS TS CS WK CK PK KS

Figure 2. Distribution of microplastics in collected fish samples from Swat and Kabul Rivers.

FTIR analysis

Microplastics, verified through microscopy under all conditions, underwent ATR-FTIR analysis for identification of chemical structure. Two types of polymers, polyethylene (PE) and polypropylene (PP), were confirmed. PE was the predominant polymer (52%), followed by PP (27%), while other polymers were not identified by ATR-FTIR. Key PE confirmation included absorption bands at 2,914.8 cm⁻¹ and 2,847.7 cm⁻¹, corresponding to methylene (-CH₂) stretches. The FTIR fingerprint region (600–1,400 cm⁻¹) revealed characteristic PE absorbance bands at 2,914 cm⁻¹ and 2,847 cm⁻¹. The FTIR spectra confirming polyethylene (PE) and Polypropylene (PP) type of microplastics are shown in Figures 4 and 5 respectively.

The FTIR spectrum of PP in literature presents a shoulder at 2,875 cm⁻¹, and the asymmetric and symmetric in-plane C–H (-CH₃) at 1455 cm⁻¹ and a shoulder at 1,358 cm⁻¹. As observed in Figure 5, FTIR spectra of MPs showed bands at 2,875 cm⁻¹ and C–H (-CH₃) stretching near 1,455 cm⁻¹ to confirm that it is a PP.

Conclusion

The findings from this study confirmed the presence of MP pollution in both Swat and Kabul rivers. The identification of MPs in the GIT of fish strongly implies their ingestion, potentially raising concerns for the well-being of organisms and the overall health of ecosystem. While the origins of these MPs in both rivers remain undisclosed, plausible contributors encompass urban runoff, agricultural practices, and plastic waste stemming from human activities. Consequently, further investigation is imperative to unveil the precise origins of these MPs and to formulate effective strategies aimed at mitigating the prevalence of MPs in these river systems. FTIR spectra analysis indicated that the predominant MPs discovered in the GIT of fish in both Swat and Kabul rivers were identified as PE and PP. Microplastics, mainly in fragment form, primarily originated from secondary sources, confirming their association with PE and PP plastic types. Moreover, the intricate interplay between MPs pollution, aquatic life, and environmental stability requires comprehensive exploration for devising well-informed conservation measures. Additionally, collaborative efforts involving scientific research, policy implementation, and public awareness campaigns are crucial in fostering a sustainable and unpolluted aquatic environment for both present and future generations.

Conflict of Interest

The authors declare no conflict of interest.

Funding

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Figure 3. Microplastics identified under stereomicroscope.
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Figure 4. FTIR spectra confirming polyethylene (PE) type of microplastics.

Figure 5. FTIR spectra confirming polypropylene (PP) type of microplastics.

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