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BARRAGES INFLUENCING MICROPLASTICS DISTRIBUTION AND INGESTION; A CASE STUDY

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Abstract: Plastic pollution is becoming a serious environmental concern because of extensive plastic use worldwide. Before being dumped into the marine environment, these anthropogenic polymers also affect freshwater ecosystems. Although extensive research has been done to evaluate the level and distribution of these anthropogenic polymers but the effect of physical structures like barrages has never been accounted so far. This study is the first of its kind to highlight the role of river barrages in determining the distribution of microplastics (MPs) and affecting their ingestion among different fish species. This study was conducted on Balloki Barrage located on Ravi River, receiving sewage water from different populous cities of Pakistan including Lahore and Sheikhupura. Water (*n=6*), sediment (*n=6*) and fish (*n=28*) samples were collected from both the upstream and downstream of the barrages to establish the relationship between MPs ingestion, fish feeding habit and the pollution level in environmental matrices. On the other hand, the relative abundance of fibers in all the fish species was 57% followed by sheets 34% and fragments 8%. Omnivore fish species were found to have more MPs than herbivores and carnivores irrespective of their size and weight. This study proved that the barrages serve an important function in determining the MPs distribution and their consequent ingestion in fish. A proper consideration must be given to the effects of these structures when evaluating plastic pollution in any riverine body.

Keywords: Microplastics, plastic pollution, barrages, fish ingestion, Pakistan

1. INTRODUCTION

Plastic is a fabricated polymer made through polymerization of oil and gas (Cole et al., 2011). Properties like durability, bio-inertness and lightweight, make it user friendly that is why its production has increased many folds (Napper et al., 2015). Nowadays, it is also used for insulating electrical cables and for designing lighter weight vehicles (Nielsen et al., 2020). Only a small portion of the used plastic items is recycled, while the rest ends up in landfills or in waterways (Naqash et al., 2020). Whilst the use of plastic is extensive, this has been a serious environmental concern (Cole et al., 2011). Uncontrolled disposal of waste and its mismanagement leads to large amount of plastic waste in every environmental media (Bergmann et al., 2015). Half of the plastic ending in oceans does not remain intact but degrades with the passage of time into different size fractions and lose its structural integrity (Güven et al., 2017). The particles having size less than 5mm are categorized into microplastics. These MPs have different densities depending upon their composition. Polyethylene terephthalate (PET) and polyvinylchloride (PVC) have higher density than seawater and sink to the bottom of water channels while low-density plastic particles float on the surface of water (Anderson et al. 2016; Do Sul & Costa 2014).

Once in the water, these tiny plastic particles become available to aquatic organisms ranging from lower to higher trophic level (Morgana et al., 2018). Fish ingest these MPs either directly or indirectly by eating zooplanktons (Güven et al., 2017). After ingestion, the harmful chemicals either used in polymerization process or adsorbed to the surface of MPs get released in the body of the organism (Anbumani & Kakkar, 2018; Brennecke et al., 2016; Naqash et al., 2020). Different negative impacts have been reported in different organisms due to ingestion of these MPs like blockage of gastrointestinal tract, respiration, and reproduction problems (Anbumani & Kakkar, 2018; Pellini et al., 2018; Yuan et al., 2019). The ingested MPs itself are either passed out of the body through feces or enter in the body tissues (Phillips & Bonner, 2015). During the transportation of MPs, they encounter many physical structures of which barrages are the most common. MPs are often discussed in terms of potential sources, distribution across the water channels and their ingestion by aquatic biota but the role of these physical structures in creating temporary reservoirs and affecting the MPs distribution has never been considered. Moreover, off taking canals from these structures also divert a considerable amount of plastic waste from their classical tract considered to be from terrestrial to the marine environment. The amount of plastic diverted by these canals again reaches terrestrial environment as the water is used for agricultural purposes.

Research has been conducted to assess the presence and abundance of microplastics in different parts of the world (Barletta et al., 2019; Dharmadasa et al., 2019; Nabizadeh et al., 2019; Xiong et al., 2018; Ng & Obbard, 2006). The MPs detected in these studies are of primary and secondary origin with different chemical composition (Eerkes-Medrano et al. 2015). Although Pakistan is lacking extensive research work in this area, but some studies are documented for terrestrial and aquatic ecosystems recently (Irfan et al., 2020a; Irfan et al., 2020b; Rafique et al., 2020). However, these studies do not include any evidence of MPs ingestion in aquatic fauna and its correlation with the prevailing MPs concentrations. Moreover, how these barriers affect their bioavailability is also an unanswered question till now.

This study was designed to quantify and classify prevailing MPs concentrations around Balloki Barrage located on Ravi River (Fig. 1). Additionally, the hypothesis that water reservoir formed by the barrage would affect the distribution of MPs in the water and sediments. The concentrations in water and sediments were then further correlated with ingested MPs detected within fish samples to further elaborate the hypothesis. Hence this paper not only summarizes the pollution level but also how it is being influenced by these manmade structures to divert or control the river flow.

2. MATERIAL AND METHODS

2.1. Ravi River and Balloki Barrage

Ravi River is a trans-boundary river that flows through India and Pakistan. In addition to plastic waste dumped by India, it also receives sewage drains from Lahore; a cosmopolitan, commercial, and industrial hub, consisting of a large amount of plastic waste. Balloki Barrage is located downstream to District Lahore where two link canals from Chenab River also discharge their water into Ravi River, hence further increasing the pollution load in the river.

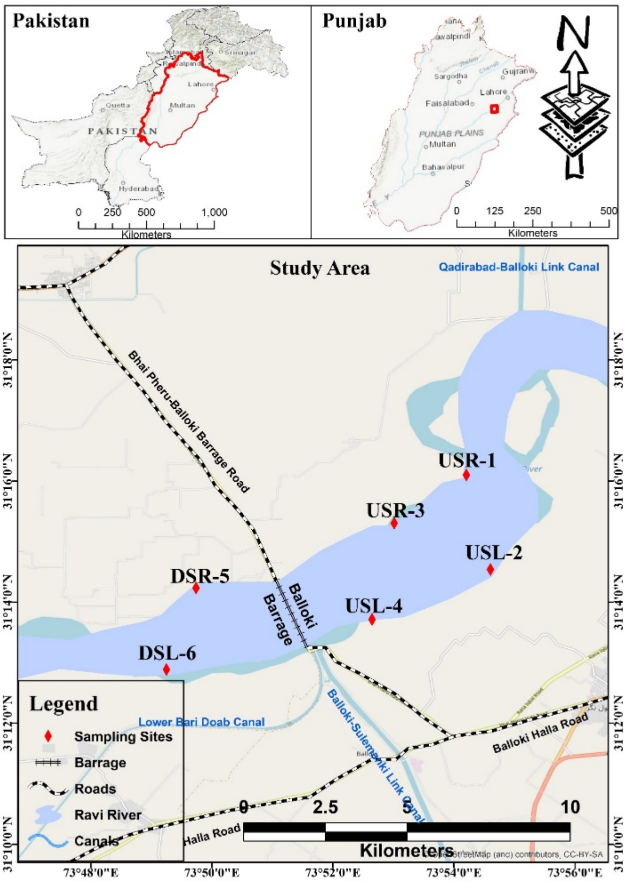

Figure 1. Study area map showing Ravi River, Balloki Barrage and sampling sites

This structure was originally built in 1915 by the British Government but it was upgraded again in 2014 as an Asian Development Project. The total length of the barrages is almost 0.5 km provide enough area for a big reservoir to be formed. Due to the large volume stored in the reservoir, sometimes it is also referred to Balloki Dam.

2.2. Sampling and pre-processing

A total of six sampling sites *(n=6)* were selected in the upstream and downstream of Balloki

Barrage (Fig. 1). Three different types of samples i.e., surface water (n=6), sediment (n=6) and fish (n=28) were collected from each site. Sampling methodologies described by Irfan et al., (2020a) were used for water and sediments while fishes were collected using a cast net having 1 cm mesh size and a circumference of 30 ft. All the samples were collected in clean glass bottles however for the fish samples 70% ethanol was added in the bottles (McNeish et al., 2018). Bottles were then covered with aluminum foil, tightly closed with lids, and labeled accordingly.

2.3. Processing of samples

The samples were stored and processed in the environmental toxicology lab of College of Earth and Environmental Sciences, University of the Punjab, Lahore, Pakistan. The fish samples were dissected carefully, and their gut content were weighed. The weighed gut contents were subjected to 10% KOH digestion in 3:1 (volume: weight) ratio as KOH is effective in dissolving tissues (Hermsen et al., 2017). The beakers were covered with aluminum foil and placed in an oven at 65°C for a week. Some partially digested samples were heated at 65°C on a hot plate until they started boiling before the density separation (Rodrigues et al., 2018). Cleaning of water and sediment samples was performed according to the protocol given by Irfan et al., (2020a). Light density particles including MPs were separated from digested samples using Sediment Microplastic Isolation units (SMI) (Irfan et al., 2020a; Coppock et al., 2017). Saturated saline solution (NaCl) was added in the SMI unit followed by the sample in a ratio of 2:1 (Nor & Obbard et al., 2014).

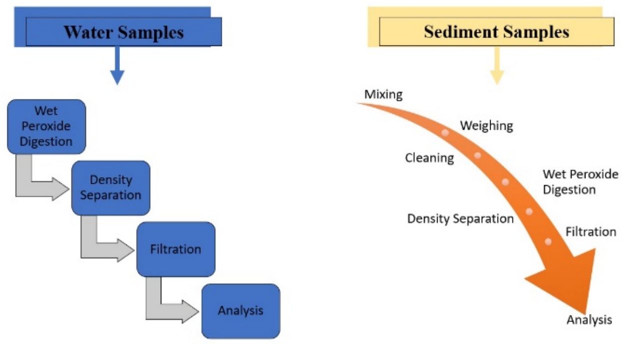

Figure 2. Schematic representation of water and sediments processing methodology.

Prior to use, the saline solution was passed through 10µm filter paper to avoid any contamination (Coppock et al., 2017). SMI units were covered with aluminum foil and left uninterrupted for 24h to allow the settling. After that, the supernatant above the check valve was poured in a glass beaker and filtered through a series of 300, 150 and 50µm sieves. The sieves were then preserved for further analysis. An overview of the adopted methodologies for water and sediments is provided in Figure 2.

2.4. MPs analysis

For the identification of MPs, the larger sieves of 300µm were observed under a stereomicroscope (IM-SZ-500, IRMECO GmbH, Germany) whereas smaller sieves of 150µm and 50µm were observed under digital microscope (Kooletron UM12C, MUS-TECH Electronics, China). Both the instruments were connected to a computer where pictures were captured and stored. In addition to three size categories, the observed MPs were subdivided into five different classes based on their shape i.e., fragments, sheets, fibers, foams and beads (Song et al., 2015). The final concentrations of MPs were calculated in terms of MPs/L, MPs/kg and MPs/individual for water, sediment and fish samples, respectively.

2.5. Laboratory quality control

All the surfaces and equipment were cleaned with 70% ethanol before starting analysis (Quinn et al., 2017). Dissection tools were also cleaned with 70% ethanol every time before and after dissecting the fish samples. The sieves were kept covered with aluminum foil before and after the filtration. Glass beakers and density separators were also covered with aluminum foil to prevent atmospheric contamination (Su et al. 2016). Similarly, distilled water, salt solution, reagents and chemicals were also filtered through 10µm filter papers to prevent contamination. Procedural blanks were also run to check the atmospheric contamination. A total of six wet filters paper were placed randomly in the laboratory for 24 hours and observed under microscope. No MPs contamination was recorded in any of the blanks.

2.6. Statistical analysis

Significance of correlation was checked between MPs concentrations in water, sediments and fish using MS Excel 2019. Origin Pro 2016 was used for the graphical illustrations.

3. RESULTS AND DISCUSSION

MPs were found in all the observed samples irrespective of their point of collection. An average of 0.80 ± 1.99 MPs/L, 135.37 ± 277.5 MPs/kg and 23.12 ± 33.07 MPs/individual were detected in water, sediment and fish samples, respectively. The prevailing

MPs concentration in surface water was comparable to Rawal Lake where 1.42MPs/L were reported (Irfan et al. 2020b). A brief comparison of our finding with some previously published reports in given in Table 1. The potential sources of these MPs in Ravi River are the anthropogenic activities in the surrounding cities like in Lahore and Sheikhupura. Lahore is one of the most populous cities of Pakistan with 12 million people and thousands of industries. The untreated effluent and sewerage water discharge from these industries has turned the river into a dumping ground (The NEWS, 2020). This situation is comparable with Raritan River, USA, assimilating discharge from various wastewater treatment plants (WWTPs) around New Jersey (Estahbanati & Fahrenfeld, 2016). The only exception for the Ravi River is that it mostly receives untreated wastewater. In addition to average MPs concentrations, the samples collected downstream to the barrages consisted of a significantly lower number of MPs. This is due to the formation of a small reservoir in the upstream of barrages where the velocity of water is significantly low, and majority of the MPs are suspended or settled in this area. This difference can even be observed across this upstream reservoir. Sampling points near to the terminal points of reservoir show high MPs concentrations both in water and shore sediments while those located in the middle broader section show lower concentrations. Hence, it can be comprehended that due to lower water velocity in the middle section of reservoir, most of the MPs are settled in core sediments and only the remaining fraction is available in surface water or shore sediments.

In our findings, around 100-415 MPs/kg were detected in the sediment samples, comparable to previously published finding from Lake Bolsena (112 MPs/kg) and Lake Chiusi (234 MPs/kg) in Italy (Fischer et al., 2016). These results are also comparale to the Taihu Lake, China (234.6 MPs/kg) surrounded by 20 million people and a large number the anthropogenic activities in its surroundings (Su et al., 2016). A similar study conducted by Irfan et al., (2020b) on Rawal Lake, Islamabad, Pakistan also showed quite similar results with an average of 104 MPs/kg. Further comparison with literature is shown in Table 1.

The distribution trend in sediment samples was found to be consistent with the surface water samples. The samples collected near to high velocity area consisted higher concentrations as compared to the lower velocity section of the reservoir. However, the MPs concentrations in the downstream sediment samples were comparatively higher than the upstream samples. The phenomena of turbulence can better interpret this difference because while passing through barrages the water becomes quite turbulent and combined with its high velocity it favors the deposition on the shore sediments. That is why, usually no deposition is possible in the core sediments due to high turbulence and velocity in the downstream area.

Table 1. Comparison of present findings with recently published studies

Matrix	Country/Site	Concentration	Reference
Water	Pakistan	1.99 ±0.80 MPs/L	**Current study**
	China	22 ± 5 MPs/L	Wang et al., (2021)
	Poland	1.5 MPs/L	Sekudewicz et al., (2021)
	Australia	0.40 ±0.27 MPs/L	Nan et al., (2020)
	Indonesia	5.85 ±3.28 MPs/L	Alam et al., (2019)
Sediments	Pakistan	277.5±135.37 MPs/kg	**Current study**
	China	1600 ±191 MPs/kg	Zhang et al., (2020)
	Iran	519.6 ± 1024.2 MPs/kg	Rasta et al., (2020)
	Algeria	649.33 ± 184.02 MPs/kg	Tata et al., (2020)
	Indonesia	3.03±1.59 MPs/kg	Alam et al. (2019)
Fish	Pakistan	33.07±23.11 MPs/individual	**Current study**
	South Korea	8.3 ± 6.0 MPs/individual	Park et al., (2020)
	China	5.3 ± 2.4 MPs/individual	Su et al., (2019)
	Hong Kong	2.4±2.3 MPs/individual	Chan et al., (2019)
	Argentina	12.1±6.2 MPs/individual	Arias et al., (2019)
	Argentina	19.2±18.9 MPs/individual	Pazos et al., (2017)
	Gulf of Mexico	4.6±3.9 MPs/individual	Phillips & Bonner (2015)

Besides the particles floating in water and deposited in the sediments, we also found them in the fish gut contents. MPs in water are usually ingested by fish (Horton et al., 2017; Murphy et al., 2017) and particularly the areas under human influence are potential sites for the ingestion (Silva-Cavalcanti et al., 2017; Peters & Bratton, 2016; Dris et al., 2015). Several international studies have confirmed the presence of MPs in different aquatic organisms including fish (De Vries et al., 2020; Huang et al., 2020; Merga et al., 2020; Park et al., 2020; Burkhardt-Holm & N'Guyen 2019; Karbalaei et al., 2019; Nie et al., 2019; Abbasi et al., 2018; Ferreira et al., 2018; Foley et al., 2018). The concentrations found the present study (23.12±33.07 MPs/individual) are comparable to some previously published findings (Table 1).

This overall trend is comparable to early published studies including Morgana et al., (2018), Wang et al., (2017), Su et al., (2016) and Zhao et al., (2014). The fibers mostly come from the washing of clothes, textile industries, breakdown of fishing nets, mismanagement at landfill sites or through some other non-point sources (Alam et al., 2019; Dris et al., 2015). Usually, ingestion of MPs is related to the extent of exposure, as they look like the food particles, but they can also be indirectly ingested when fish feeds on its prey who already have ingested MPs (Nie et al., 2019; Ory et al., 2017; Pazos et al., 2017). Territory and feeding habit of a fish also play an important role in determining the amount of ingested plastic particles (Murphy et al., 2017). In terms of habitat, bottom dwelling fish species ingest more MPs than mid-water or surface-water dewellers (Zheng et al., 2019). Similarly, omnivorous fish species ingest more MPs than that of carnivorousand herbivorous fish species because of their diverse feeding range consisting of planktons, benthic organisms, algae and humus (Garcia et al., 2020). Similar trends were found in our findings where omnivorous *Labeo calbasu* and *Cirrhinus mrigala* had the highest number of ingested MPs as compared to the other fish species. Some previously published studies also reported that omnivorous fish species ingested more MPs as compared to other fish species (Wang et al., 2020; Zheng et al., 2019; Mizraji et al., 2017). Additionally, we found no correlation between the fish size and ingested MPs concentrations.

Mastacembelus armatus of 30cm length was found to have 26 Mps as compared to *Osteobrama cotio* (8cm) which was having 57 MPs. Hence, the concentration of ingested microplastics is dependent upon the feeding nature of the fish species and not on the size of fish. These results coincide with the previous published findings of De Vries et al., (2020) and Güven et al., (2017) who also could not prove any correlation between the fish size and ingested MPs.

3.1 MPs shapes and their relative abundance

Fibers were abundant in surface water with relative abundance of 57% followed by fragments (21%), sheets (21%) and beads (1%). Similarly, in sediment samples, the relative proportion of fibers, sheets, fragments, foams and beads was found to be 55.5%, 20%, 19.8%, 2.7% and 1.2%, respectively. Representative MPs from each category are shown in Figure 3.

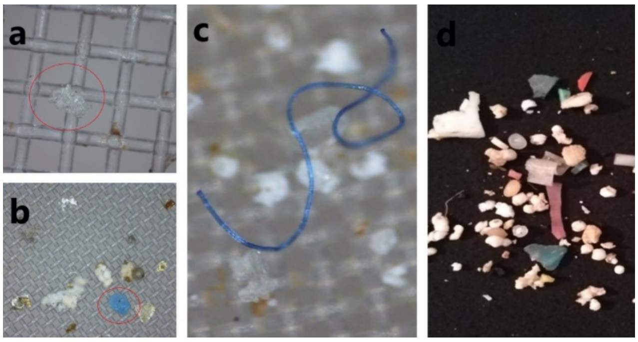

Figure 3. Different MPs detected from the samples (a) foam (b) fragment (c) fiber (d) randomly collected MPs

Fragments are mostly formed due to the breakdown of packaging material, large plastic products including plastic bottles (Di & Wang, 2018; Horton et al., 2017) hence being most prevalent. MPs ingested by fish also coincide with the surface water data in terms of defined shapes indicating a direct connection between the fish and surrounding environment. Lusher et al., (2013) and Jabeen et al., (2017) also reported highest proportion of fibers among the ingested MPs. Fibers are morphologically identical to algae that is why they are largely consumed by omnivores and herbivore fish species (Wang et al., 2020). Furthermore, due to their ductile nature, fibers are ingested by the aquatic organisms at lower trophic level, and they also get mixed with the sediments more often. Both characteristics increase the proportion of fibers among the ingested MPs (Silva-Cavalcanti et al., 2017).

4. CONCLUSION

Our findings show that MPs were present not only in water and sediment but in fish species as well. Fibers were dominant in all the samples followed by fragments and sheets. Although, concentration of MPs in all environmental matrices was found to be consistent with the previous published literature but we found a significant decline in MPs concentrations while moving from upstream to the downstream of barrage depicting settling of MPs in the reservoir. Moreover, it was noticed that more turbulence and high velocity of water tends into higher MPs concentrations in surface water and sediments while in comparatively low velocity areas fish is usually exposed to more suspended MPs. The types of MPs ingested by the fish correspond perfectly with the prevailing types in surface water. It was proven that apart from diverting MPs load from marine environment to the terrestrial environment, barrages also play a key role in determining their distribution and bioavailability. All these factors combined, make barrages as potentially important structures to be considered when dealing with riverine plastic pollution. There is need to study temporal variations of MPs in river system to determine the effects of changing climatic conditions. It is also recommended to conduct studies to evaluate proportion of MPs diverted into agricultural lands by barrages and their consequences on the human population.

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