Assessment of heavy metals in two commercial fish species collected from river Jhelum, Pakistan.

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Abstract- Mining, waste disposal, and fossil fuel combustions are key factors that are polluting the aquatic environment rapidly. Bioaccumulation and toxicity of heavy metals are major problems of the aquatic environment which change the physicochemical properties of water which is hazardous for aquatic organisms especially fish because heavy metals develop oxidative stress in fish bodies.

This study was designed to determine the levels of heavy metals cadmium, nickel, arsenic, and lead in the liver and gills of two most important edible fish species; *Cirrhinus mrigala* and *Bagarius bagarius*, taken from the Jhelum River district Khushab, Punjab, Pakistan. In the current study, to check the presence of heavy metals the process of wet/acid digestion was implemented to prepare samples of fish liver and gills then the prepared samples were analyzed using an atomic absorption spectrometer. Heavy metal lead was recorded in the highest amount in the gills of *Cirrhinus mrigala* and *Bagarius bagarius* with the values (0.69µg/g) and (0.77µg/g) respectively. The order of heavy metals examined in the gills of *Cirrhinus mrigala* was Pb>Ni>As>Cd. The sequence of heavy metals examined in the gills of *Bagarius bagarius* was Pb>As≥Ni>Cd. Heavy metal nickel was recorded in the highest concentration in the liver of *Cirrhinus mrigala* (0.63µg/g) and in *Bagarius bagarius* arsenic concentration was highest with the value of (0.56µg/g). The order of heavy metals examined in liver of *Cirrhinus mrigala* was Ni>Cd>As>Pb while the sequence of heavy metals examined in the liver of *Bagarius bagarius* was As>Ni>Cd>Pb. Overall, all the heavy metals values were exceeding the WHO limits imposing an alarming situation. Bioaccumulation of such heavy metals in fish is dangerous because when humans consume contaminated fish on regular bases they also bioaccumulate heavy metals which may lead to different health issues, particularly endocrine disruption. Proper disposal of waste and recycling along with chemical precipitation and ion exchange are some measures to avoid harmful consequences of heavy metals contaminations.

Keywords- Bio-accumulation, *Bagarius bagarius, Cirrhinus mrigala,* Heavy metals

I. Introduction

Fish contains important nutrients like proteins, lipids, vitamins (A, B, and D), and minerals (Phosphorus, Magnesium, Iron, Iodine, and Calcium) (Golden *et al*., 2016). Its high polyunsaturated fatty acid (omega-3 polyunsaturated) content help to lower blood cholesterol levels (Huynh *et al*., 2009). Fish provide essential elements such as 85 mg/kg zinc, 350 mg/kg iron, and 84g/kg calcium (Glover-Amengor *et al*. 2012).

Cirrhinus mrigala is a riverine carp and locally it is known as Mori/Morakh. It is a detritivorous fish. Based on its quick growth, it is used in polyculture along with other carp (Tiwari *et al*., 2015). *Cirrhinus mrigala* is one of the three main carps native to India and is found across Asia (Talukder *et al*., 2017). *Bagarius bagarius* is native to Nepal's, India's, Pakistan's, and Myanmar's rivers and this fish is commonly known as Gonch. *Bagarius bagarius* is a widely distributed fish family in South Asian countries, including four species in particular; *Bagarius bagarius, Bagarius rutilus, Bagarius suchus, and Bagarius yarrelli. Bagarius bagarius* is the most common of the four species in Indian stream systems (Roberts, 1983). It is a carnivorous fish that play a vital role in the river food chain.

Heavy Metals:

Heavy metals have an atomic density of more than 5g/cm³ or five times that of water but the density of heavy metal isn't a consideration, the metal's electronegativity (the ability to receive and give electrons to other elements in a complex) does as toxicity rises with the rise in electronegativity. Heavy metals inevitable use in modern industry and agriculture has prompted widespread acceptance in the climate, raising worries about potential health and environmental impacts. Cadmium compounds are used in PVC products, dyes, polymers and nickel-cadmium batteries. Cadmium induces hepatocyte vascular degeneration, pancreatic cell necrosis and fatty alterations in peripancreatic hepatocytes (Dangre *et al.,* 2010). The kidney is the principal target organ for cadmium toxicity and chronic exposure in animals, with different degrees of renal impairment (Kumar and Sing 2010). Cadmium can also cause oxidative stress and free radicals indirectly by overproducing reactive oxygen species (ROS) or consuming cell cancer prevention agent levels (Cuypers *et al*., 2010). Arsenic affected fish face gastrointestinal, osmosis, skin, liver, and kidney damage. Arsenic alternate the biochemical nature of fish blood in aquatic climates (Authman *et al*., 2015). In humans, short-term exposure to arsenic causes abdominal pain, diarrhea, and muscle weakness, while long-term exposure causes skin abnormalities and cancer (Bosch *et al*., 2016). Batteries, old lead pipes, lead-based paints, and leaded gas have all increased lead fixation. Lead binds to metallothionein, transferrin, calmodulin, and calcium-ATPase that suppresses erythrocyte growth and heme synthesis enzymes, as well as lipid peroxidation enzymes. Lead alters the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in tissues and organs (Cogun and Sahin, 2012). Lead has been shown to impact the neurological, digestive, reproductive, circulatory, and immunological systems of animals (Al-Balawi *et al.*, 2013). The growing usage and manufacturing of nickel-containing products in many sectors and processes have increased nickel content in aquatic bodies. This is owing to the usage of nickel in metallurgical facilities, silver refineries and copper or brass plating businesses (Javed and Usmani 2019). Around 75% of nickel is utilized in alloy manufacturing.

II. Materials and methods

The study was conducted on freshwater species *Cirrhinus mrigala* and *Bagarius bagarius.* Fish samples were collected from September 2021 to December 2021 from four different sites of river Jhelum's downstream at district Khushab, Punjab, Pakistan. The total number of samples for each fish specie was 20 per month (fortnight sampling; 10 samples each fortnight). The average total length of *Cirrhinus mrigala* was 41.68±1.23cm with an average weight of 734.5±61.78g and the average total length of *Bagarius bagarius* was 81.99±2.71cm with an average weight of 2191.25±243.10g

Acid/wet Digestion:

For acid/wet digestion 1g sample of liver and gill was put in a 30ml flask separately then 5ml concentrated Nitric acid was added to the flask and the hot plate was turned on. When the mixture turned deep brown (approximately after five minutes for 1 gram sample) a 5ml solution of 3 parts concentrated Perchloric acid was added and this acid mixture oxidized the sample solution. The 1g sample digestion was done in 45mins then the contents of the flask were poured into a 125ml separatory funnel containing 20ml of triple distilled (deionized) water. The flask was rinsed with 10ml triple distilled water and then in a separatory funnel with 10ml of methyl-iso-butyl ketone (MIBK) was added and thoroughly shaken upon separation of the two phases, the lower aqueous phase diluted to any degree and samples were stored in air-tight bottles.

Heavy metals analysis:

The prepared samples were studied using atomic absorption spectroscopy for the heavy metal analysis and T-test was applied to the results obtained from the absorption spectroscopy. The significance of the differences between results was $p \leq 0.01$.

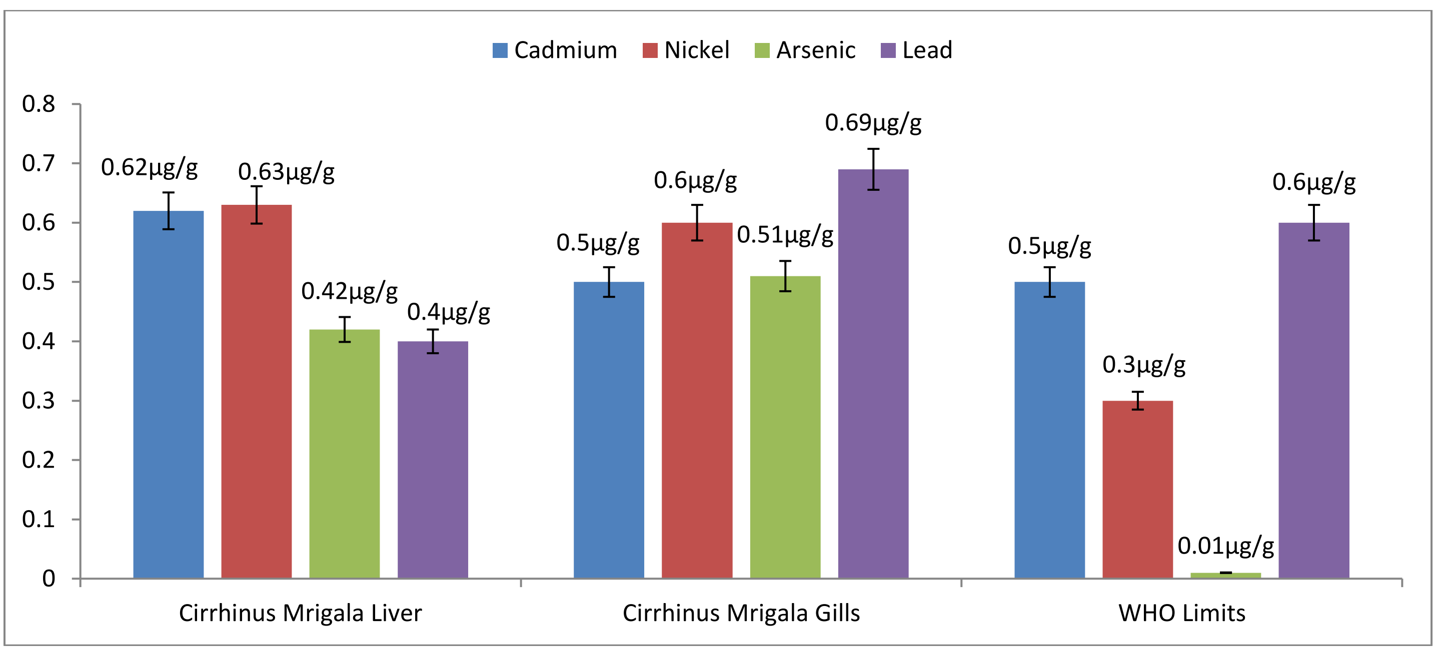
Fig I. Sampling stations.

III. Results

Obtained data showed that in most cases, the amount of heavy metals in target organs (liver and gills) was equal to or higher than the permissible limits set by WHO.

Table I. Heavy metals in liver and gills of *Cirrhinus mrigala* (N=80):

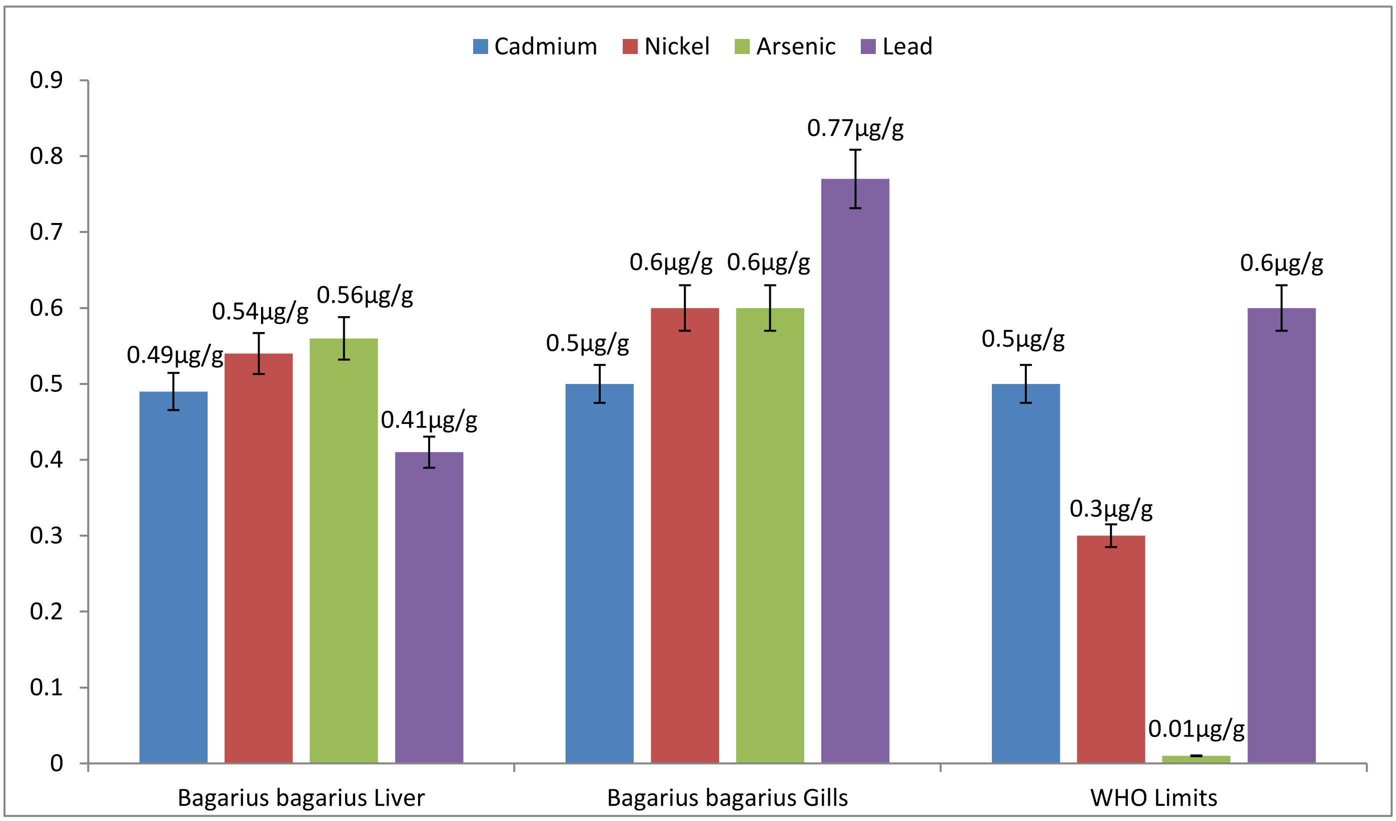
Heavy Metal	Liver	Gills	WHO Limits	Heavy metals in River Water	Significant Value:p
Cadmium	0.62±0.16 µg/g	0.50±0.12 µg/g	0.50 µg/g	2.0 µg/g	**p<0.001**
Nickel	0.63±0.14 µg/g	0.60±0.12 µg/g	0.30 µg/g	2.2 µg/g	**p<0.001**
Arsenic	0.42±0.15 µg/g	0.51±0.14 µg/g	0.01 µg/g	1.9 µg/g	**p<0.001**
Lead	0.40±0.15 µg/g	0.69±0.13 µg/g	0.60 µg/g	2.1 µg/g	**p<0.001**



Graph I. Heavy metals in liver and gills of *Cirrhinus mrigala*

Table II. Heavy metals in liver and gills of *Bagarius bagarius* (N=80)

Heavy Metals	Liver	Gills	WHO limits	Heavy metals in River Water	Significant Value:p
Cadmium	0.49±0.14 µg/g	0.50±0.12 µg/g	0.50 µg/g	2.0 µg/g	**p<0.001**
Nickel	0.54±0.15 µg/g	0.60±0.12 µg/g	0.30 µg/g	2.2 µg/g	**p<0.001**
Arsenic	0.56±0.15 µg/g	0.60±0.15 µg/g	0.01 µg/g	1.9 µg/g	**p<0.001**
Lead	0.41±0.14 µg/g	0.77±0.14 µg/g	0.60 µg/g	2.1 µg/g	**p<0.001**



Graph II. Heavy metals in liver and gills of *Bagarius bagarius*

Comparison of heavy metals in liver of *Cirrhinus mrigala* (N=80) and *Bagarius bagarius* (N=80).

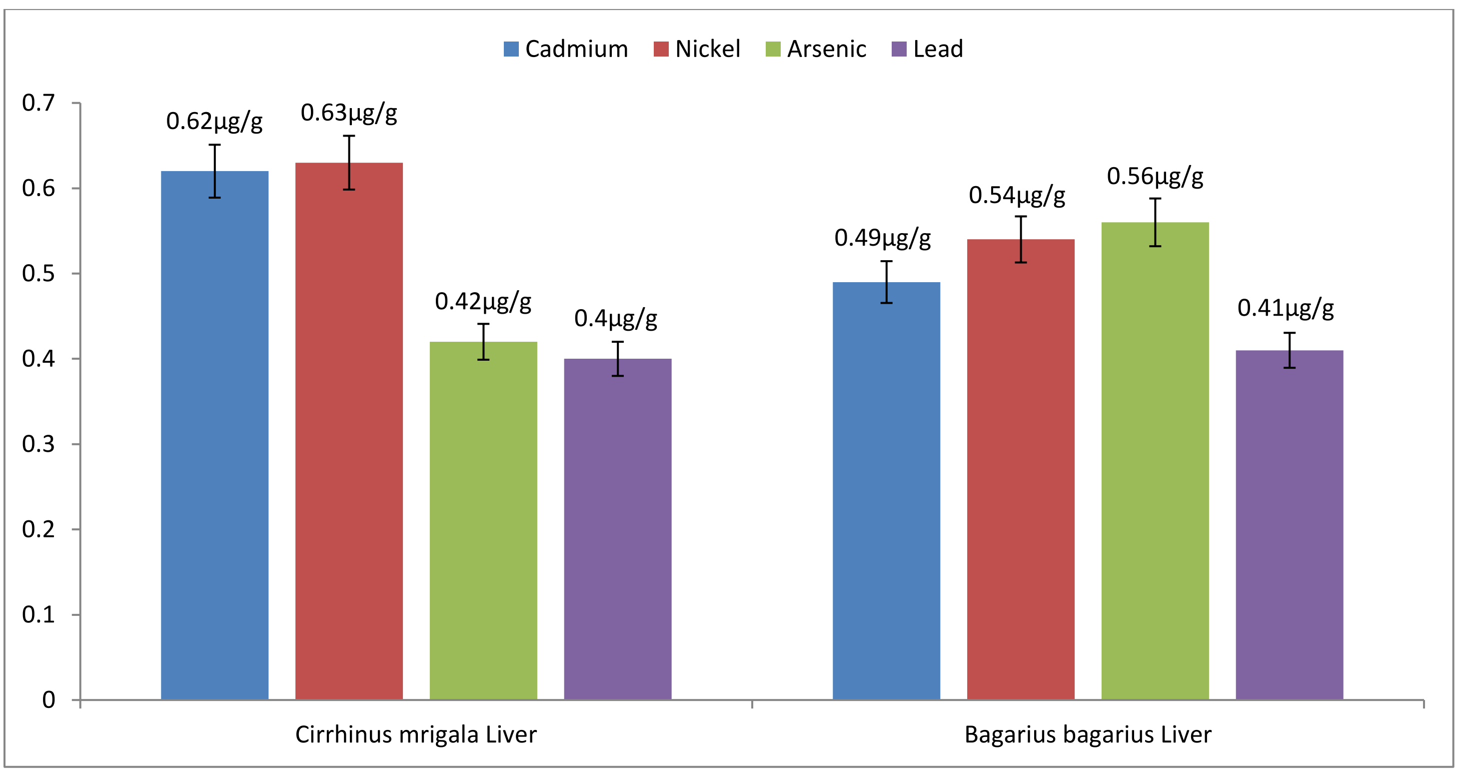
The investigation showed that in the liver of *Cirrhinus mrigala* there were high levels of heavy metal cadmium with the value of 0.62 µg/g and heavy metal nickel's amount was 0.63µg/g, both values were higher than the recommended values set by WHO for cadmium (0.5µg/g) and nickel (0.6µg/g). While value observed for arsenic in the liver of *Bagarius bagarius* was (0.56µg/g) which was also exceeding the WHO standard limits of arsenic (0.01µg/g). Arsenic's value for *Cirrhinus mrigala* (0.42µg/g) was also higher than WHO limits. The values observed for heavy metal lead in both fish were almost the same with a value of 0.40µg/g in *Cirrhinus mrigala* liver and 0.41µg/g in the liver of *Bagarius bagarius* which was less than the standard limits of WHO (0.6µg/g).

The sequence of the heavy metal in *Cirrhinus mrigala* fish liver was recorded as Ni>Cd>As>Pb.

While the sequence of the heavy metal in *Bagarius bagarius* liver was recorded as As>Ni>Cd>Pb.

Table III. Comparison of heavy metals in the liver of *Cirrhinus mrigala* (N=80) and *Bagarius bagarius* (N=80).

Heavy Metals	Fish Species	Liver	WHO limits	Significant Value:p
Cadmium	***Cirrhinus mrigala***	0.62±0.16 µg/g	0.50 µg/g	**p<0.001**
	Bagarius bagarius	0.49±0.14 µg/g		
Nickel	***Cirrhinus mrigala***	0.63±0.14 µg/g	0.30 µg/g	**p<0.001**
	Bagarius bagarius	0.54±0.15 µg/g		
Arsenic	***Cirrhinus mrigala***	0.42±0.15 µg/g	0.01 µg/g	**p<0.001**
	Bagarius bagarius	0.56±0.15 µg/g		
Lead	***Cirrhinus mrigala***	0.40±0.15 µg/g	0.60 µg/g	**p<0.001**
	Bagarius bagarius	0.41±0.14 µg/g		



Graph III. Comparison of heavy metals in the liver of *Cirrhinus mrigala* (N=80) and *Bagarius bagarius* (N=80).

Comparison of heavy metals in gills of *Cirrhinus mrigala* (N=80) and *Bagarius bagarius* (N=80).

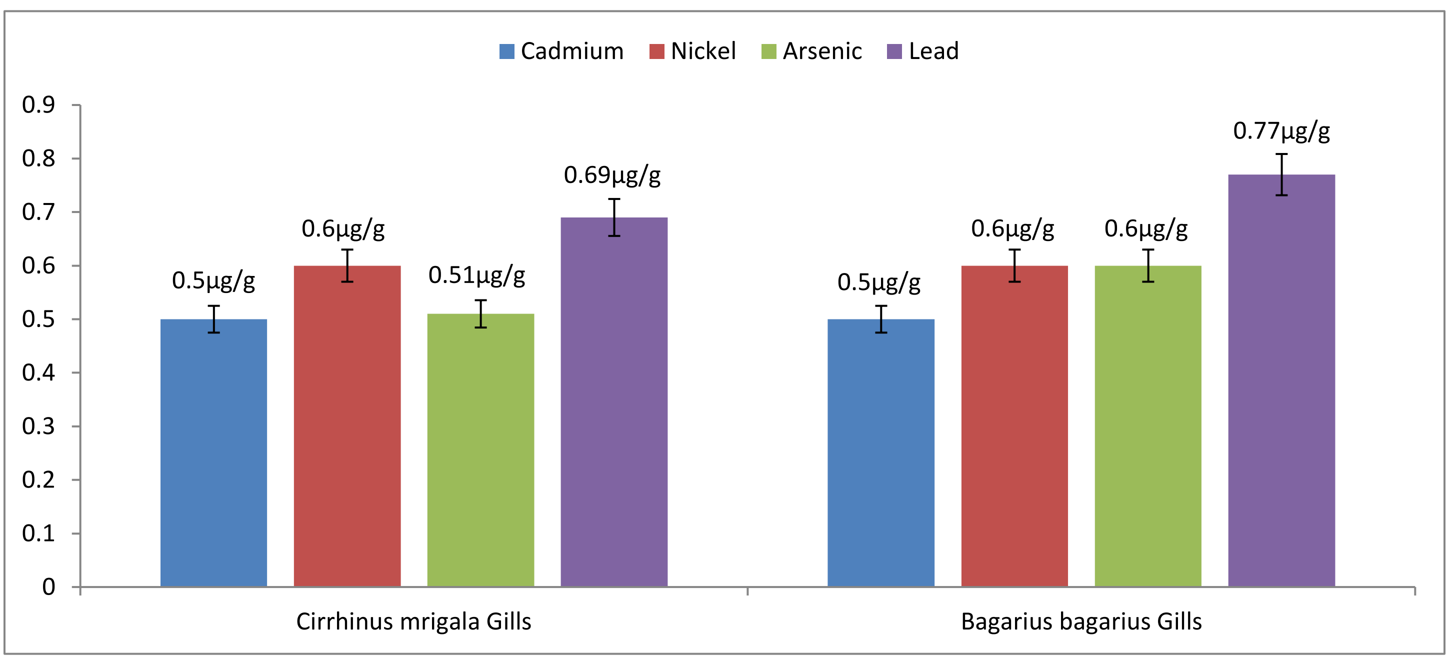
Current study showed that in the gills of both the fish *Cirrhinus mrigala* and *Bagarius bagarius* the most abundant heavy metal was lead and its value was (0.69µg/g) in the gills of *Cirrhinus mrigala* and (0.77µg/g) in the gills of *Bagarius bagarius* and these values exceeding the acceptable range set by WHO for lead (0.6µg/g). Arsenic and Nickel were the second most abundant heavy metal found in the gills of *Bagarius bagarius* with values of (0.6 µg/g & 0.6 µg/g). The heavy metal cadmium and nickel were recorded in the same quantity in the gills of both the fish i.e. cadmium in *Cirrhinus mrigala* and *Bagarius bagarius* was (0.50µg/g) and nickel in the gills of both fish was (0.60µg/g). The values of cadmium were equal to the standard WHO values of cadmium (0.5 µg/g).

The heavy metal sequence in *Cirrhinus mrigala* gills was as follows; Pb>Ni>As>Cd.

And the sequence for *Bagarius bagarius* gills the heavy metal was as follows; Pb>As≥Ni>Cd.

Table IV. Comparison of heavy metals in gills of *Cirrhinus mrigala* (N=80) and *Bagarius bagarius* (N=80).

Heavy Metal	Fish Species	Gills	WHO limits	Significant Value:p
Cadmium	***Cirrhinus mrigala***	0.50±0.12 µg/g	0.50 µg/g	**p<0.001**
	Bagarius bagarius	0.50±0.12 µg/g		
Nickel	***Cirrhinus mrigala***	0.60±0.12 µg/g	0.30 µg/g	**p<0.001**
	Bagarius bagarius	0.60±0.12 µg/g		
Arsenic	***Cirrhinus mrigala***	0.51±0.14 µg/g	0.01 µg/g	**p<0.001**
	Bagarius bagarius	0.60±0.15 µg/g		
Lead	***Cirrhinus mrigala***	0.69±0.13 µg/g	0.60 µg/g	**p<0.001**
	Bagarius bagarius	0.77±0.14 µg/g		



Graph IV. Comparison of heavy metals in gills of *Cirrhinus mrigala* (N=80) and *Bagarius bagarius* (N=80).

IV. Discussion

The results showed that heavy metal accumulation in Gibel carp was tissue-specific, i.e. each tissue had a different capacity for collecting heavy metal. In general, all tissues had large amounts of Zn and Cu, but much lower amounts of Ni, Cr, Pb, and Cd. Such wide discrepancies in heavy metal accumulation may be explained, at least in part, by their reliance on different types of heavy metals, such as essential metals and non-essential metals (Roy 2010).

Current research showed that the amount of heavy metals in both fish varied to some extent in the liver and gills. This might be owing to a distinct accumulation pattern, as the liver is the location of detoxification, which is why it contains the most heavy metals. The concentrations of all metals were considerably higher ($p<0.001$). The amount of Lead, Cadmium and Nickel was high, which could be due to a variety of factors, including the fact that *Cirrhinus mrigala* feeds on dead decaying matter, which is present on the river's bottom, and heavy metals accumulate at the river's bed, increasing the likelihood that heavy metals transfer from dead organic matter to the fish. *Bagarius bagarius*, on the other hand, is a carnivorous fish, therefore the fish may easily pass from small fish and prawns to this fish, which feeds on them.

Cadmium and Lead are non-essential heavy metals that are toxic. These metals have no known biological function in the animal body and are toxic in even minimal concentrations. Their toxicity stems in part from competition for binding sites with essential metals, as well as interference with sulfhydryl groups, which are necessary for enzyme and structural protein function (Da Silva *et al*., 2005).

Meanwhile, current study reveals the heavy metal lead concentrations in the gills of both experimental fish (*Cirrhinus mrigala* & *Bagarius bagarius*) were greater than other metal concentrations. The high amount of lead is due to the widespread use of lead-acid batteries, such as car batteries, and the ease with which they can be recharged. When these batteries are disposed of and thrown into the sewerage system, they eventually run down to streams, lakes, and rivers and settle at the bottom, and because the *Cirrhinus mrigala* is a bottom feeder, it can easily uptake heavy metals from there.

Heavy metal concentrations in *Cyprinus carpio* samples were seen to decrease in sequence, with Fe>Cu> Pb>Ni>Cr>Cd in the muscle and stomach-intestine; Fe>Cu>Ni>Pb>Cr>Cd in the gill, heart, and liver; and Fe>Cu>Ni>Pb>Cr>Cd in the air sac. The kidney, together with the gills and the intestines, are essential for excretion and maintaining body fluid equilibrium. The kidney, in addition to producing urine, serves as an excretory pathway for the metabolites of a variety of xenobiotics to which the fish may be exposed. Metal concentrations were identified in both fish's livers in the sequence Pb>As>Cr, and in both fish's muscles in the order Pb>Cr>As (Kousar *et al*., 2014).

In the current investigation, the most prevalent heavy metal in the gills of both the fish *Cirrhinus mrigala* and *Bagarius bagarius* was lead, which was (0.69µg/g) in the gills of *Cirrhinus mrigala* and (0.77µg/g) in the gills of *Bagarius bagarius*, and both values were greater than the WHO tolerable level for lead, which is (0.6µg/g). Pb>Ni>As>Cd was the heavy metal sequence found in *Cirrhinus mrigala* gills and the heavy metal sequence in *Bagarius bagarius* gills was as follows: Pb>As>Ni>Cd. (Nawaz *et al*., 2010) evaluated the Ravi River's contamination caused by industrial waste and human activities. The investigations looked at As, Cd, Pb, Hg, and Zn. Higher amounts of As (35.74-45.33 ppm), Cd (0.35-0.45 ppm), Pb (2.1-3.0 ppm), and Hg (83.03-92.35 ppm) were shown to be related to normal electrolyte levels. In this study, Cd (0.3 µg/g), Pb (0.3-7.2 µg/g), Ni (0.3-2.7 µg/g), and As (0.7-2.18 µg/g) values were examined. This investigation showed that consuming *Cirrhinus mrigala* and *Bagarius bagarius* from the River Jhelum might be detrimental to consumers since heavy metal concentrations were similar to or greater than the WHO-authorized limits for human consumption.

V. Conclusion:

All organisms are extremely sensitive to the toxicity of heavy metals such as arsenic, cadmium, lead, and nickel. When the acceptable levels of heavy metals exceed the limits set by WHO, they can cause serious poisoning. Heavy metals can enter the water through drainage, air, and soil erosion. As the concentration of heavy metals in aquatic ecosystems rises, these elements bio-accumulate in the food chain, causing toxicity in animals, including fish.

The selection of these two fish for investigation was based on their popularity among consumers due to their exquisite flavor and abundance in the Jhelum River. *Cirrhinus mrigala* fish consume detritus, decomposing organic waste, zooplankton, and phytoplankton. While the *Bagarius bagarius* is a fish that feeds on small insects, prawns, and frogs. Therefore, both fish are at a high risk of receiving heavy metals, however, *Cirrhinus mrigala* is more susceptible to absorbing heavy metals since heavy metals settle near the bottom of the river. In the case of the *Bagarius bagarius*, which feeds on insects and frogs, heavy metals can easily enter its body through the food chain. All the heavy metals enter the river Jhelum because there are places in Khushab city where these metals are produced in various ways, such as when cadmium and lead are thrown into the city's wastewater system during the repair and maintenance of automobile batteries. Both of these metals are abundant in the environment due to their widespread use. While nickel and arsenic are utilized and dumped into the river via the sewage system, since they are employed in the manufacturing of alloys and as a doping agent, respectively. The amounts of heavy metals in River Jhelum fish species differed. Lead accumulated greater in the gills of both *Bagarius bagarius* and *Cirrhinus mrigala* overall. Because fish gills are constantly in touch with polluted river water, the likelihood of heavy metals absorption via fish gills is significantly greater. The amount of lead in the gills of *Cirrhinus mrigala* and *Bagarius bagarius* was 0.69µg/g and 0.77µg/g receptively and both these values were higher than the recommended values of WHO. While in both fish gills, the second heavy metal was nickel and its value was 0.6µg/g for *Cirrhinus mrigala* and (0.6 µg/g) for *Bagarius bagarius.* While in the case of *Cirrhinus mrigala* liver, nickel was the most abundant heavy metal having a value of 0.63µg/g and in *Bagarius bagarius* liver, arsenic was in high value the liver with 0.56µg/g. All the metal concentrations were discovered to be higher than the recommended values set by WHO.

If we compare these results with other researchers the presence and values of heavy metals in the fish were equal to some studies or even higher and this is a very alarming situation because natural water sources are getting more and more polluted day by day due to rapid industrialization and domestic untreated waste and the presence of these heavy metals in the fish body can easily pose a bad impact to humans when humans consume such contaminated fish and can cause a lot of health issues like renal failure, cancer, hormonal imbalance via mimicry and skeletal problems.

Suggestions:

As pollution levels rise, strong measures must be implemented to protect natural water bodies and aquatic life from catastrophic aquatic contamination. Industrial effluents and domestic waste should be appropriately disposed of. Some of the ways to lessen the burden of heavy metal are as follows and we must use them for a better future. Chemical precipitation is a method in which effluents are precipitated as insoluble carbonates or sulfides and are then removed by sedimentation and filtration. This is one of the efficient removal processes for heavy metal effluent treatments as it is easy to use and has a low operational cost. The ion exchange method can also be used in which heavy metal ions of contaminated water attach to resin beads and metal-free water is released to the sewage system and resin beads are safely moved to burying sites. Other methods can also be used to treat heavy metal-contaminated water before dumping it into natural resources like adsorption, membrane filtration, reverse osmosis, solvent extraction, and electrochemical treatment.

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