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Assessing Heavy Metal Contamination in Fish: Impact on Human Health and Ecosystem Integrity

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SUMMARY

An assessment of heavy metals (lead, titanium, aluminum, barium, calcium, chromium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, sulphur, silicon, and zinc) in the organs (kidney, liver and muscle) of two fish species (*Channa punctatus* and *Oreochromis aureus*) was carried out from the sampling sites of Chenab River near Head Trimmu, Pakistan. The analysis was performed by using the inductively coupled plasma mass spectrometry. To identify the significant variances ($p<0.05$) in heavy metals, a two-way analysis of variance (ANOVA) was performed. The kidneys of *C. punctatus* and *O. aureus*, respectively, contained high concentrations of calcium and aluminum. Sulphur and calcium both had an impact on the muscle cells of *C. punctatus* and *O. aureus* while the aluminum concentrations were found to be highest in the liver of both species. It was found that both fish species had high levels of calcium, aluminum, and phosphorus absorption. Based on the target hazard quotients (THQ), the health hazards associated with eating fish for adults and children that were both non-carcinogenic and carcinogenic were evaluated. The results of the health risk assessment indicated that all the elements except chromium, iron and manganese, had safe THQ values for fish consumption. Based on the results, it is proposed that the heavy metal intoxication of the freshwater ecosystems should be strictly regulated; otherwise, the fate of the fish population and the people who consume it may lead to incalculable loss.

Keywords: Estimated Daily Intake, Fish, Head Trimmu, Heavy metals, Risk Assessment, Target hazard quotient

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INTRODUCTION

Heavy metals have drawn a lot of interest in aquatic environments because of their toxicity and accumulation in biota. Despite their relatively low concentrations, some of these metals are harmful to living things while some metals are naturally occurring, biologically necessary components that only become poisonous in very high quantities in aquatic ecosystems (Orosun et al., 2016). Heavy metal toxicity in fish can affect physiological processes, individual development rates, reproduction, and mortality (Per-Arne et al., 1997; Nwadozie, 1998; Gillanders et al., 2001; Yujun et al., 2011; Ruqia et al., 2015). Three routes exist for heavy metals to enter fish bodies: through the digestive system, the body surface, or the gills (Per-Arne et al., 1997). In general, fish is regarded as one of the most affordable and healthy sources of proteins because it contains a higher percentage of cysteine in its amino acid composition than the majority of other protein sources (William, 2008). Fish flesh is advised for heart health due to its reduced total and saturated fat content. Fish flesh is abundant in long-chain omega-3 fatty acids, notably Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)(Per-Arne et al., 1997; Nwadozie, 1998; Gillanderset al., 2001; Yujun et al., 2011; Ruqia et al., 2015;William, 2008). Additionally, it contains essential trace minerals and proteins.

O. aureus (Tilapia) and *C. punctatus* (Dola) are the most popular fish with reduced fat content and higher levels of these heart-healthy nutrients (William, 2008). Tilapia and Dola are among the fish that Asians most frequently eat because of their flavor and affordability. As a result of studies, heavy metals impact the physiological balance and other fish physiological systems as well. The fish's physical and chemical habitat is apparent to alter how quickly these heavy elements are absorbed into their body.The results of being exposed to any harmful material depend on the quantity, duration, mode, and manner of exposure, as well as customs, features and whether additional substances are present (Per-Arne et al., 1997; Nwadozie, 1998; Gillanderset al., 2001; Yujun et al., 2011).

Accumulation of different heavy metals varies with the species of the fish. The chemical properties of the water from which fish samples were taken, ecological variations, fish metabolism, and eating habits have all been implicated in the heavy metal concentrations in fish (Yujun et al., 2011; Al-Salehand Shinwari, 2002; Ozturk et al., 2009; Yilmaz, 2003). Fish bioaccumulation of metals can be used as an indicator of the level of metal contamination in aquatic environments [Yujun et al., 2011; Forstner and Wittmann, 1981; Jimohet al., 2004; Karadede and Unlu, 2007; Chi et al., 2007).

For some dams and rivers in Asia, including Trimmu dam the levels of concentration of hazardous and non-biodegradable heavy metals in fish samples have been documented. In this article, head Trimmu sampling sites have been studied to assess the heavy metal contamination in freshwater fishes. Both fish species sourced from head Trimmu were found to be contaminated with heavy metals, and further analysis revealed that the fish livers exhibited significantly higher concentrations of these heavy metals compared to their muscular tissues (meat).

Even though fish have significant nutritional components, their capacity to bio-accumulate harmful and non-biodegradable heavy metals in their edible body parts requires a sufficient measurement of the metal levels in them at regular intervals to ensure the safety of the consumers. The goals of this study were to compare the levels of heavy metal bioaccumulation in the two fish species, to examine if there were any notable differences in the levels of each metal, and to calculate the potential risks to human health from consuming these local fish.

MATERIALS AND METHODS

AREA OF STUDY

The Punjab and Khyber Pakhtunkhwa provinces make up the majority of the Chenab River's hydrographic basin, which is situated in northeast Pakistan. The Chenab River basin is included in the Subtropical Continental Highlands phytogeographic zone. The area has a subtropical climate with four distinct seasons, including hot summers and somewhat frigid winters. Dense forests once covered the upper portion of the Chenab River valley, near its source, but throughout time, these lands were transformed for a variety of purposes, including plantations of commodities like sugarcane and rice fields. Large areas of land are used for cattle ranching and rice farming in the centre of the basin. The lower section is heavily industrialized and populated, especially close to urban centres, with a concentration of different industries.

However, the increased industrialization and urbanization in the Chenab River territory have led to several environmental issues. The ecosystem of the river has undergone significant stress as a result of water withdrawal for domestic and industrial uses as well as the discharge of untreated domestic and industrial sewage. The site for sampling was chosen near the head of Trimmu in the Chenab River.

COLLECTION AND PROCESSING OF SAMPLES

Cast nets, hand nets, and drag nets were used to randomly catch ten members of each fish species from the head Trimmu area between January and March 2022.The fish samples (*Channa punctata* and *Oreochromis aureus*) were kept in ice boxes and brought to the lab. Samples were kept there at -20°C till analysis. Each fish was measured for length and weight as it arrived. Each fish specimen's kidney, liver, and muscle were extracted with care and kept in pristine polyethylene tubes.

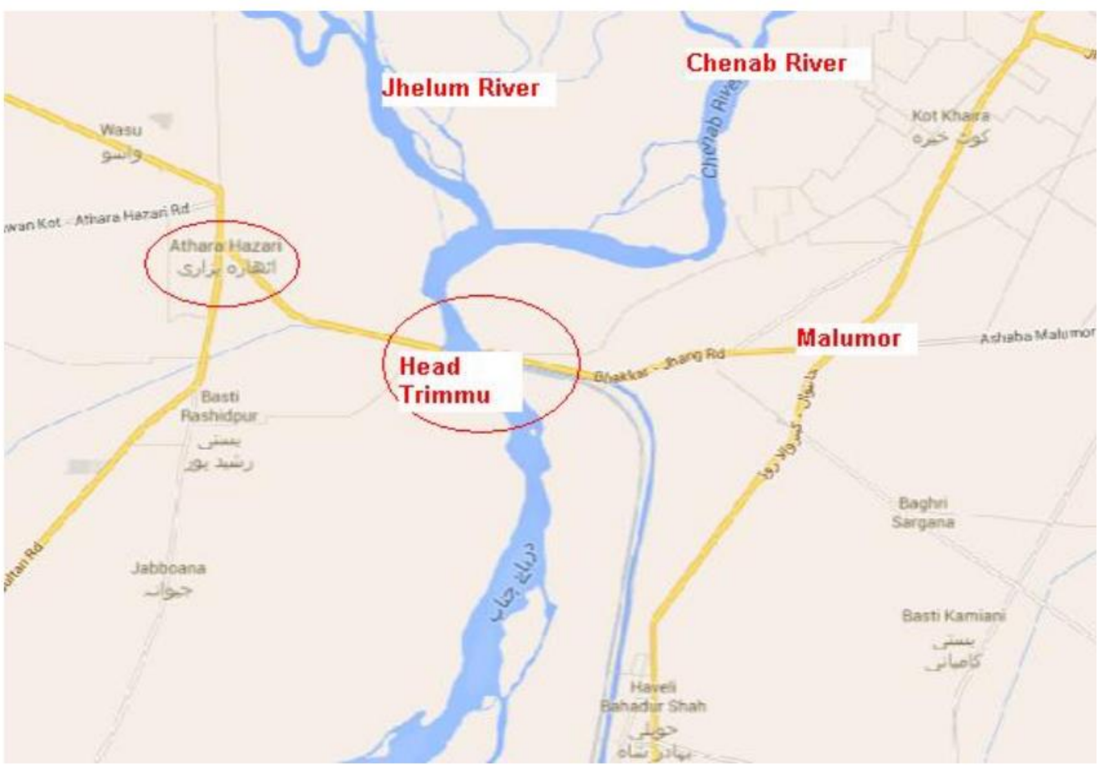


Figure 1: Pictorial map showing the selective sampling site

PROCEDURE FOR WET DIGESTION

The following actions were taken to wet digest the samples:

a. Weighing: Each liver, kidney and muscle sample was accurately weighed at about 250 mg before being put into a separate glass tube.

b. Nitric Acid Addition: Five millilitres of strong, ultra-pure nitric acid were added to each glass tube containing the liver sample.

c. Glass tubes were heated for three hours at 120°C to undergo "digestion" which aids in dissolving the components and disintegrating the organic material.

d. Transfer and Cooling: The digested samples were poured into polypropylene flasks after cooling.

e. Dilution: Each solution was diluted to a final volume of 30 ml using de-ionized water. This dilution guarantees that the element concentrations are within the analytical instrument's detection range.

INSTRUMENTAL ANALYSIS

Following the digestion of liver, kidney, and muscle samples, an instrumental analysis was performed using an ICP-OES (inductively coupled plasma optical emission spectrometer; a Spectro Model Ciros CCD from Kleve, Germany) with the collaboration of Pakistan Institute of Nuclear Science and Technology (PINSTech). The spectrometer was equipped with a cross-flow nebulizer, a glass double pass spray chamber, and a quartz torch featuring a quartz injector tube with a 2 mm inner diameter.

CALIBRATION AND QUALITY CONTROL

Prior to sample analysis, the instrument's performance was optimized by changing variables such the torch alignment, RF power, and nebulizer gas flow rate in line with the instructions provided by the manufacturer.

The amounts of trace elements in the samples of Dola and Blue tilapia were discovered using this wet digestion technique and subsequent ICP-OES analysis. The bioaccumulation of metals and the state of these fish species' health in the head Trimmu area are both useful information offered by this analytical approach.

STATISTIC EVALUATION

The detected metal concentrations were statistically analyzed by using SPSS version 16.0. The data were summarized and described using descriptive statistics, and significance was determined using ANOVA (Analysis of Variance). To grasp the central tendency and variability of the results, the trace element data was presented as mean standard deviation (Table 3, 4 and 5)

Using Microsoft Excel for Windows 10, graphs were made for a better interpretation of the data.

CHEMICAL EVALUATION

The health concerns linked to metal exposure have been assessed using a variety of assessment methods. The Estimated Daily Intake (EDI) approach is one important tool for determining the quantity of pollutants ingested daily. The amount of potentially harmful substances found in food and daily food consumption are directly correlated with the EDI (Table 6)

To calculate the EDI for heavy metals in humans, the following formula is used:

$$\mathrm{EDI} = \frac{\mathrm{C \times C_{ons}}}{\mathrm{Bw}} \quad \ldots\ldots\ldots (1)$$

Here, C stands for the concentration of heavy metals in the fish body, C cons for the average daily intake of fish in the region, and Bw for the person's weight.
The Hazard Quotient (HQ), which contrasts the EDI with the Reference Dose (RfD), is another significant parameter in the assessment of health risks. This is how the HQ is determined:

$$\mathrm{HQ = RfD / EDI} \ldots\ldots.. (2)$$

Negative health impacts are unlikely if the HQ value is found to be less than 1. However, if the HQ value is greater than 1, it indicates that exposure to heavy metals may have harmful consequences on one's health. Understanding the possible risks linked to metal exposure and directing suitable risk management methods depend greatly on this investigation.

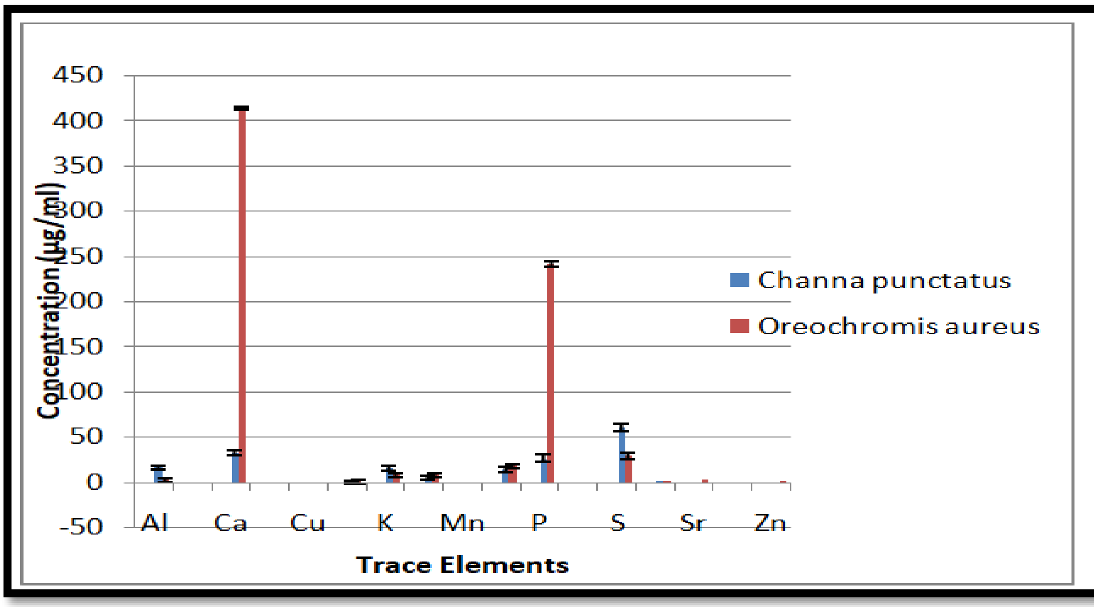
RESULTS

BIO-CONCENTRATION OF HEAVY METALS IN *C. PUNCTATUS* AND *O. AUREUS*

The study conducted a comprehensive analysis of the bio-concentration of 17 distinct heavy metals in two fish species, *C. punctatus* and *O. aureus*, shedding light on their potential environmental exposure and uptake patterns. Among the metals studied, zinc was a common presence in both species, albeit with variations in concentration. Notably, *C. punctatus* exhibited a zinc concentration of 0.293 µg/ml in the liver, 0.355µg/ml in the kidney, and 0.20 µg/ml in muscles. In contrast, *O. aureus* showed higher levels, particularly in the muscles with 0.39 µg/ml, followed by the liver at 0.4 µg/ml, and the kidney at 0.28 µg/ml. Titanium was another metal found in both species, with the liver of *C. punctatus* containing the highest concentration at 0.27 µg/ml. *O. aureus* displayed greater levels, particularly in the liver, which registered 0.42 µg/ml of titanium. Silicon was notably present in the muscles of both species, with *C. punctatus* at 0.88 µg/ml and *O. aureus* at 0.89µg/ml, but *O. aureus* showcased higher silicon contents in its tissues. Sulfur concentrations were markedly higher in the muscles of both species, with *C. punctatus* at 60.12 µg/ml and *O. aureus* at 28.75µg/ml, while sulfur content in the kidneys was lower. Lead was an intriguing discovery, exclusively detected in *O. aureus* kidneys, albeit at a relatively low concentration of 0.2µg/ml. Strontium was detected in trace amounts, primarily in *O. aureus* muscles, with a concentration of 0.08 µg/ml. Phosphorus, on the other hand, was widespread in all tissues of both species, reaching its highest concentrations in the muscles, where *C. punctatus* had 25.63 µg/ml, and *O. aureus* had a substantially higher 242.05 µg/ml.

Sodium was universally present in all tissues, with the muscles harboring the most substantial amounts, measured at 13.91 µg/ml in *C. punctatus* and 17.62 µg/ml in *O. aureus*. Manganese was found in limited quantities in both species, with *O. aureus* exhibiting slightly higher levels. Magnesium was pervasive in all tissues of both species, with the muscles containing the highest concentrations, reaching 4.46 µg/ml in *C. punctatus* and 8.02 µg/ml in *O. aureus*. Potassium was also prevalent in all tissues, with the muscles holding the highest levels, at 14.94 µg/ml for *C. punctatus* and 7.87 µg/ml for *O. aureus*. Iron was detected in the liver and kidneys of both species, with

O. aureus showcasing higher liver iron content. Copper and chromium were present in low concentrations across all tissues of both species. Calcium was identified in all tissues, with the kidneys of *C. punctatus* containing the highest concentrations at 157.05 µg/ml, while *O. aureus* muscles exhibited notably high levels, with 414.02 µg/ml. Barium was found in modest quantities in various tissues, with *O. aureus* liver displaying the highest concentration at 0.19 µg/ml. Aluminum was detected in all tissues, with the muscles of both species containing the highest amounts, measuring 16.32 µg/ml in *C. punctatus* and 22.11 µg/ml in *O. aureus*. The study's findings provide valuable insights into the bio-concentration patterns of these heavy metals, underscoring the importance of considering both species and tissue-specific variations in environmental assessments. (Table 1, 2, Figures 2, 3 and 4).



Figue 2: Volume of heavy metals in the kidney of *C. punctatus* and *O. aureus*.

HEAVY METALS CORRELATION BETWEEN ORGANS OF *C. PUNCTATUS* AND *O. AUREUS*

The study examined the correlation of heavy metal concentrations among the organs of *C. punctatus and O. aureus*, shedding light on the intricate patterns of metal accumulation. Notably, in the kidneys, there were significant differences ($p<0.05$) in the levels of Aluminum, Calcium, Copper, Magnesium, Phosphorus, Sulphur, Barium, Silicon, Strontium, Potassium, and Titanium between the two species, underscoring the distinct metal uptake dynamics in this vital organ. Conversely, elements such as Chromium, Iron, Manganese, Sodium, Lead, and Zinc did not exhibit significant differences ($p>0.05$) between the species in kidney tissues (Table 3).

In the liver, significant variations ($p<0.05$) were observed in Aluminum, Barium, Iron, Calcium, Potassium, Manganese, Magnesium, Sodium, Silicon, Sulphur, and Strontium concentrations, highlighting diverse hepatic metal accumulation tendencies. Conversely, elements like Chromium, Copper, Titanium, Phosphorus, and Zinc showed no significant differences ($p>0.05$) between the two species in liver tissues (Table 4).

Finally, in the muscles, significant differences ($p<0.05$) were apparent in Aluminum, Barium, Calcium, Potassium, Magnesium, Sodium, Phosphorus, Lead, Sulphur, and Strontium levels, elucidating distinctive metal distribution patterns within this tissue. Conversely, elements such as Chromium, Iron, Copper, Silicon, Titanium, Manganese, and Zinc displayed no significant differences ($p>0.05$) between the species in muscle tissues (Table 5).

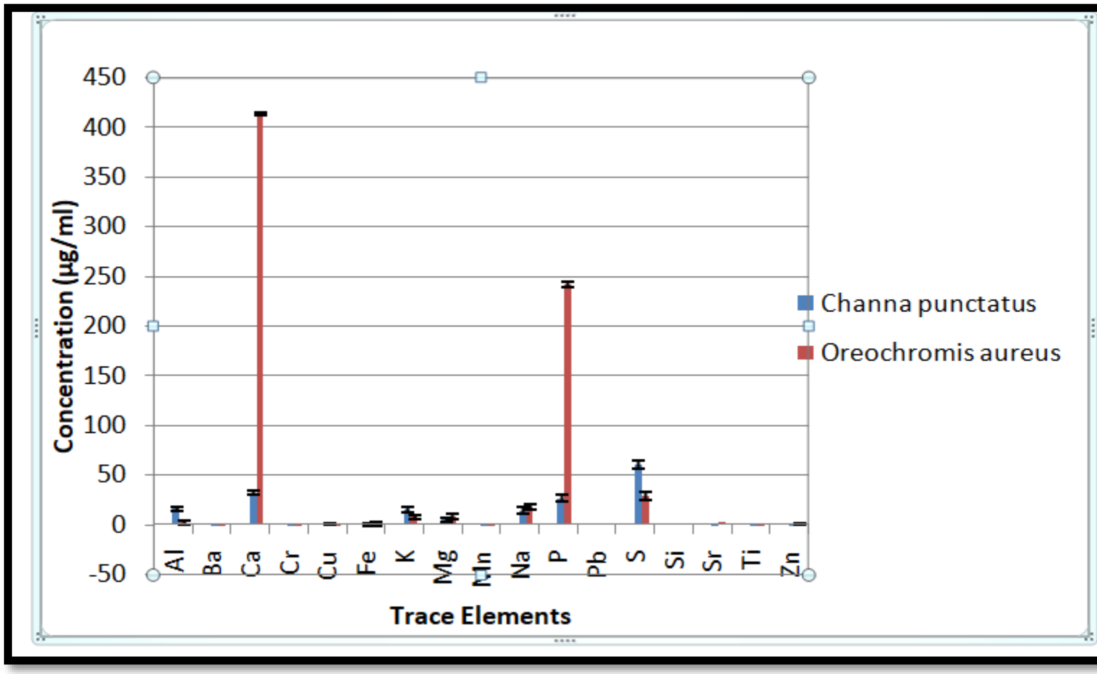


Figure 3: Volume of heavy metals in the liver of *C. punctatus* and *O. aureus*.

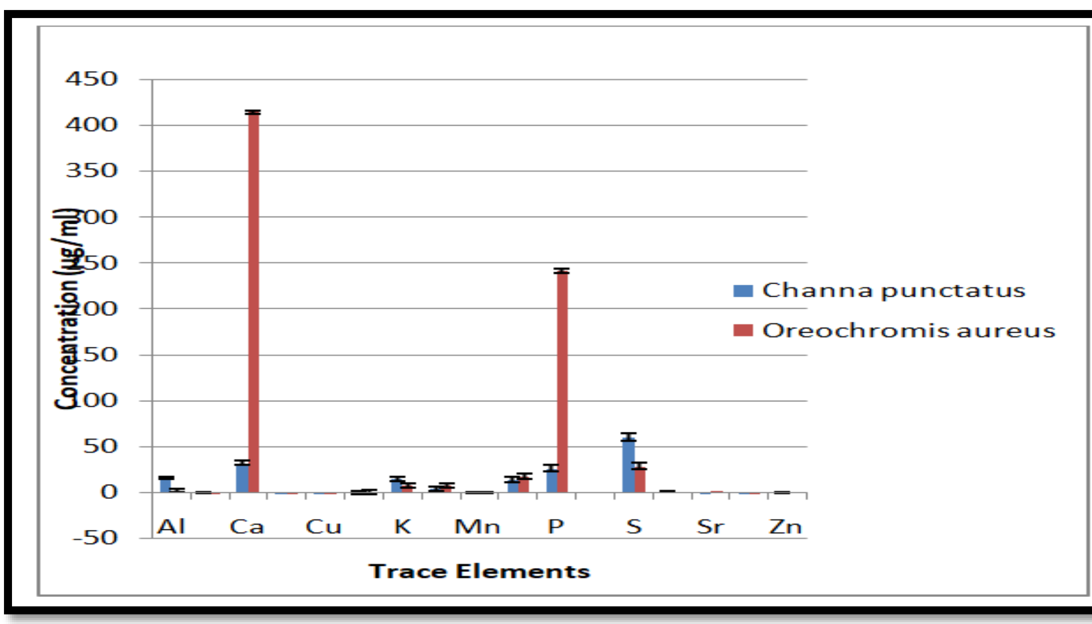


Figure 4: Volume of heavy metals in the muscle of *C. punctatus*and *O. aureus.*

These findings underscore the organ-specific variations in heavy metal concentrations within *C. punctatus and O. aureus*, emphasizing the importance of considering individual organs when assessing the bioaccumulation of these metals in aquatic organisms.

POTENTIAL ECOLOGICAL RISK

The health risk assessment findings showed that consumption of *C. punctatus* and *O. aureus* posed substantial health concerns for manganese (Mn) for both adults and children. The THQ value for iron (Fe) in adults was similarly higher than 1 when ingesting *O. aureus*. Additionally, both fish species' adult THQ values for chromium (Cr) were higher than 1, indicating serious health hazards. The detrimental health effects of chromium may be more pronounced in adults compared to children because it was shown to be safe for youngsters to consume within those limits (Table 6).

DISCUSSION

Fish that are exposed to high levels of waterborne metals can bio-accumulate these metals by absorbing them through their skin and gills, or by consuming contaminated food and water. As a result, metal levels in fish tissues may be many times higher than those found in the water (Jabeen and Chaudhry, 2010). Fe showed larger accumulation in the liver of *O. aureus*. Mn is a necessary element for both plants and animals (Anim et al., 2011), and it is largely harmless to aquatic life and may be under homeostatic control (Weiner and Geisy, 1979) and is also rarely a problem in freshwaters. Additionally, fish kidney and liver, which is the site of active metabolic activities, tends to accumulate it.

Fish liver, which has vigorous metabolic processes, accumulates Zn (Moiseenko et al., 2008; Nolan et al., 1992) effectively. Moderate quantities of zinc were identified in both fish species, with larger quantities in the liver and kidney of *O. aureus* and *C. punctatus*, respectively. According to the THQ standard value, zinc did not pose a threat to the health of human consumers. Cu is an essential element because of its significant function in biological systems (Fallah et al., 2011), and the low levels in each organ identified in the current investigation are consistent with its homeostatic control (Pyle et al., 2005). In both species, copper contents were determined to be moderate, with larger amounts being observed in the liver of *O. aureus*. Consumers of copper were not shown to be at risk for negative health impacts from copper.

Fish connected with the substrata or those that eat it had the highest liver Zn contents, which may be an indicator of recent assimilation through the digestive tract (De Souza Lima Junior et al., 2002). In comparison to the species studied in this work, higher Zn accumulation values were found in the liver of *O. aureus* and comparable values appeared in another omnivorous fish from the same site where a significant amount of organic garbage is released (Costa and Hartz, 2009).

Chromium is a non-essential element that is utilized in the production of steel, inks, and the leather industry, among various other applications (De Souza Lima Junior et al., 2002). The amounts of this metal in the fish liver found in the Chenab River were not acceptable for use by humans. Higher Cr concentrations were found in the fish species himri from the Orontes (*Carasobarbus luteus*, 1.22-2.57 g g^{1}w.w.River, Turkey, where even the locals suffer from residential and industrial sewage Wastes and agricultural operations were ruled out, and the fish were not declared safe in light of the restrictions on human consumption (Yilmaz and Dogan, 2008).

Fish species from the Chenab River have livers that had relatively high iron (Fe) buildup values. Given that the metal tends to concentrate in the sediment of the Chenab River, which contains considerable amounts of iron itself, the eating habits of both species may account for these high Fe levels. Livers of both fish species had the highest quantities of iron, with the liver of *O. aureus* having noticeably greater levels. The study indicated that iron concentrations could potentially cause hazardous health effects on human adults who consume *O. aureus*. Similar levels of Fe accumulation in the liver of *Carasobarbusluteus* (150.51-354.12 g g^{1}w.w.) in a contaminated site (Karadede and Ünlü, 2000) and *Chondrostomaregium* (185.36 g g^{1}w.w.) taken from the Atatürk Dam Lake, an unpolluted site, were found.More than 10 ppm of excess iron has been linked to hypertension, sleepiness, a rapid increase in pulse rate, and blood clotting in blood vessels.

Toxic metals Fe and Pb can be absorbed, retained, and concentrated by organisms as they move up the food chain (Kosanovic et al., 2007). An investigation conducted in Pakistan's Manchar Lake revealed higher Pb values for *Oreochromis mossambicus* (Arian et al., 2008), an omnivorous fish with a tendency towards detritivory, than for *C. voga* liver (1.44-2.19 g g1 w.w.On the other hand, lead was not found in any other organs of *O. aureus* but only found in trace amounts in the kidney. Although the study's lead content was higher than the USEPA's THQ limit, it was concluded that lead through ingestion of the relevant fish species was unlikely to have a negative impact on human health.

Lead is only found in kidney of *O. aureus*; even though the mean Pb contents are within the WHO's 0.4–0.5 ppm standard for consumable fish, Pb can bioaccumulate in humans over time if they continue to consume these fish samples. People should be concerned about this bioaccumulation because higher Pb concentrations have been shown to even result in irreversible brain damage (Bakare-Odunola, 2005; WHO, 2008; Lawrence, 2014). Lead is a compound toxin that has the potential to cause cancer in people. Autoimmunity, in which a person's immune system destroys its own cells, may also be brought on by it. Joint disorders and ailments of the kidneys, circulatory system, and neurons can result from this (Bakare-Odunola, 2005).

Chromium is a crucial trace element (Mertz, 1969) and its physiologically useable form is crucial for the metabolism of glucose. The average person is said to need around to lg each day. Chromium deficiency impairs growth and causes problems with protein, lipid, and glucose metabolism (Calabrese, 1985). Long-term exposure to Cr has also been linked to skin irritation, liver, renal, circulatory, and nerve tissue damage (Tseng, 2013; Smith et al., 1992). Chromium levels were within acceptable ranges, and in *C. punctatus*, the muscle had the largest quantities of chromium, whereas in *O. aureus*, liver had the highest chromium concentration.

Aluminium, calcium, and sulphur were all found in large proportions in the liver, kidney, and muscle of *C. punctatus*. This was different from a prior study where it was discovered that C. punctata organs contained high levels of iron (WHO, 2008; Lawrence, 2014).

The study evaluated the heavy metal content of two fish species, *C. punctatus* and *O. aureus*, as well as the water quality in aquatic habitats. Based on physicochemical characteristics, the water was determined to be adequate for fish nutrition, but some heavy metals were discovered to be above the authorized maximum levels. Both fish species from the Chenab River showed relatively high iron, manganese andchromium buildup concentrations. Given that the metal tends to concentrate in the sediment of the Chenab River, which contains considerable amounts of iron itself, the eating habits of both species may account for these high Fe levels. Livers and kidneys of both fish species had the highest quantities of Fe, Cr, and Mn and the liver of *O. aureus* having noticeably greater levels. The study indicated that iron concentrations could potentially cause hazardous health effects on adults who consume *O. aureus* and *C. pumctatus*.

Since the muscle is the main fish portion ingested by humans, many studies have examined the accumulation of heavy metals there (Meche et al., 2010; Terra et al., 2008; Yi et al., 2011), thus if they become contaminated with poisonous compounds, it could be harmful to human health. However, unlike the liver and kidney, which are organs of continuous accumulation, biotransformation, and detoxification and offers a more immediate assessment of the current environmental level of pollutants (Jaric et al., 2011), muscle is not always a good indicator of the whole body fish contamination but it is clear that fish tissues always have higher quantities of heavy metals than water does.

The higher metal concentration levels were identified in the detritivore species (*O. aureus*), which refutes the theory that fish in the upper web feeding position are more likely to accumulate more metals. This is consistent with the observation that fish ingest metals mostly by feeding in low-contaminated waterways (Farkas et al., 2003). According to Yousafzai et al. (2010), omnivore fishes (*O. aureus*) accumulated 65.2% greater metal burden than carnivore fishes (*C. punctatus*), which is consistent with our findings. Different ecological requirements, metabolic processes, and eating habits may result in a difference in the concentration of heavy metals in various fish species (Allen-Gil and Martynov, 1995). Regardless of the location, it is clear that *O. aureus* has larger concentrations of these particular heavy metals than *C. punctatus*. It is thought that this is because of the difference between their metabolisms and dietary habits. The metabolic activity of aquatic animals is widely known to be one of the most crucial aspects that significantly contribute to the buildup of heavy metals (Yujun et al., 2011; Sivaperumal et al., 2007; Kabata-Pendias, 2011). Due to its production of the metallothionein enzyme, which can bond with potentially dangerous trace metals, the liver may be a target organ for the accumulation of trace elements while the muscle tissues of freshwater fish are shielded by their skin from the hazardous substances in the environment. Little metal is transmitted from the fish's other body parts to its muscles since they cannot be pierced by foreign metals and cannot detoxify heavy metals like the liver does (Ilyas et al., 2023). Maximum concentration of heavy metals in muscle did not exceed permissible limits for both species; THQ value was lower than 1, indicating that non-carcinogenic health issues are not seen for consumers except for Cr, Mn, and Fe where THQ values were greater than 1 (THQ>1).Overall, our findings highlight the significance of taking into account any potential health hazards related to the eating of various fish species, particularly for manganese, iron, and chromium, in order to protect both adults and children's wellbeing. It was also found that a combination of elements, such as dry weather, the discharge of organic matter, and subsequently low levels of dissolved oxygen in the water, was likely the contributing causes of the severe fish kill event in the Chenab River rather than high concentrations of heavy metal pollutants in fish tissues.

CONCLUSION

Freshwater ecosystems including lakes, streams, and rivers are susceptible to heavy metal pollution as a result of human activities. In Head Trimmu, Pakistan, *C. punctatus* and *O. aureus* levels of 17 potentially hazardous heavy metals were measured as part of this experiment. The liver of *O. aureus* accumulated the highest concentrations of Zn, Mn, Fe, Cr, and Ba, whereas the kidney of *O. aureus*contained the highest concentrations of K, Cu, Ti, Si, Cr, and Al. The kidney of *C. punctatus* contained high concentrations of Cr and Ca. The muscles of *O. aureus* contained the highest concentrations of Sr, P, Na, and Mg, while the muscles of *C. punctatus* had the highest concentration of S.According to the research, *C. punctatus* and *O. aureus* are both susceptible to heavy metal contamination. A health risk assessment suggests that consuming fish captured from Head Trimmu may not be safe because of the potential risks from Cr and Fe and Mn.

Additionally, employing complex THQ parameter rather than a straightforward measure like the concentration of metals in the samples gives a better picture for assessing the health risks of heavy metals. As the THQ values for Cr, Fe, and Mn were above the threshold values for children and adults indicating that non-carcinogenic health issues are seen for consumers so consumption of these metals is not safe for humans. Therefore, the sources of heavy metal contamination in the hydrosphere should be regulated since the dangers to human health from consuming fish from this region were significantly over the level of inconsequential quantities.

FUTURE RECOMMENDATIONS

It is essential to monitor heavy metal levels continuously, enforce tight rules on trash discharge, and raise public knowledge of the dangers of heavy metal contamination if freshwater ecosystems and aquatic life are to be protected. The ecosystem's equilibrium can be preserved by using ecosystem-based river health management strategies and promoting sustainable fishing methods. Early warnings of environmental changes can be provided by bio-monitoring programmers that use indicator species, and studies on metal deposition in the food web will assist determine possible health concerns to people. Additionally, putting money into restoration projects, encouraging teamwork, and carrying out long-term studies will aid in efficient management and the preservation of these crucial ecosystems.

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Table: 1. Heavy metals concentration in different organs of *Channa punctatus* from head Trimmu.

	Muscle				Liver				Kidney			
	Muscle				Liver				Kidney			
Metal	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**
Al	16.39	1.454	14.85	18.99	14.97	3.213	9.97	19.99	35.66	5.03	28.78	44.97
Ba	0.019	0.019	0	0.01	0.011	0.027	0	0.09	0.048	0.019	0.02	0.048
Ca	31.85	31.85	25.82	35.87	8.694	3.111	4.97	13.75	157	6.86	145.9	165.01
Cr	0.043	0.043	0.01	0.08	0.033	0.022	0	0.07	0.033	0.022	0	0.07
Cu	0.069	0.069	0.01	0.2	0.084	0.06	0	0.2	0.069	0.054	0.01	0.2
Fe	1.027	1.027	0.06	2.1	4.74	1.937	1.79	6.91	1.924	0.093	1.78	0.99
K	14.96	14.96	10.97	19.99	1.812	1.267	0.08	0.09	1.294	0.276	2.09	1.95
Mg	4.468	4.468	1.76	7.09	1.952	1.085	4.11	4.02	3.187	1.828	0.27	6.09
Mn	0.048	0.048	0.02	0.08	0.055	0.025	0.01	5.8	0.293	0.505	0.04	1.7
Na	13.91	13.91	9.83	18.7	10.43	3.326	0.1	14.81	11.09	2.834	6.78	15.04
P	25.63	26.63	19.76	32.67	10.37	2.895	5.97	15.01	95.52	3.359	89.97	95.521
S	60.12	60.12	50.96	65.18	11.91	3.739	5.87	17.83	13.98	4.237	8.97	21.48
Si	0.887	0.887	0.09	2.57	0.731	0.552	0.1	1.57	1.13	0.076	0.99	1.21
Sr	0.082	0.082	0.03	0.2	0.033	0.022	0	0.07	0.4	0.288	0.09	1.2
Ti	0.21	0.21	0.03	1.01	0.279	0.423	0.03	1.4	0.2	0.317	0.01	1
Zn	0.202	0.202	0.01	1.01	0.293	0.505	0.04	1.7	0.355	0.241	0.19	1.01

Table 2: Heavy metals concentration in different organs of *Oreochromis aureus* from head Trimmu.

Metals	Muscle (µg/ml)				Liver (µg/ml)				Kidney (µg/ml)			
	Mean	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**	**Mean**	**S.D.**	**Min**	**Max**
Al	2.232	0.047	2.17	2.3	78.843	2.07	76.44	81.85	111	4.249	105.11	117.21
Ba	0.082	0.036	0.03	0.15	0.011	0.049	0.09	0.27	0.0175	0.025	0	0.08
Ca	414.027	3.332	407	417.02	26.245	1.704	23.02	28.04	4.483	2.667	0.09	7.97
Cr	0.039	0.025	0	0.08	0.06	0.026	0.02	0.1	0.043	0.021	0.01	0.08
Cu	0.06	0.028	0.02	0.1	0.127	0.024	0.09	0.16	0.169	0.084	0.09	0.38

Fe	0.578	0.0319	0.53	0.62	12.829	2.377	9.87	16.92	2.008	1.432	0.09	4.5
K	7.871	0.0324	7.82	7.92	17.221	2.273	14.09	20.98	4.253	2.406	0.08	7.06
Mg	8.023	2.34	5.06	11.01	6.858	2.191	3.76	10.06	0.927	0.124	0.71	1.15
Mn	0.103	0.252	0	0.8	0.376	0.11	0.19	0.51	0.116	0.04	0.05	0.17
Na	17.625	1.001	15.99	18.99	15.084	2.692	11.06	19.06	11.449	1.998	8.9	14.97
P	242.054	0.641	241.04	243.04	9.686	2.485	6.09	13.05	4.379	1.954	1.89	7.76
Pb	0	0	0	0	0	0	0	0	0.2	0.317	0.01	1
S	28.755	0.0508	28.69	28.85	16.423	3.486	11.21	20.71	7.554	1.98	4.99	10.09
Si	0.895	0.031	0.85	0.95	1.286	0.135	1.01	1.44	2.443	1.505	0.76	5.01
Sr	1.986	0.062	1.91	1.986	0.189	0.029	0.15	0.25	0.024	0.017	0	0.05
Ti	0.16	0.0483	0.09	0.22	0.423	0.036	0.37	0.47	23.086	2.475	19.41	26.07
Zn	0.392	0.0429	0.33	0.45	0.4	0.216	0.1	0.7	0.285	0.611	0.01	1.98
Zn	0.202	0.202	0.01	1.01	0.293	0.505	0.04	1.7	0.355	0.241	0.19	1.01

Table 3: Statistical comparison of heavy metals in kidney of *C. punctatus* and *O. aureus.*

Trace metals	F value	p (0.05)	Adjusted R^2
Al	1.308	0.00	0.986
Ba	8.971	0.008	0.296
Ca	4.297	0.00	0.996
Cr	1.008	0.329	0.000
Cu	9.849	0.006	0.318
Fe	0.034	0.855	0.054
K	14.920	0.001	0.423
Mg	15.200	0.001	0.428
Mn	0.157	0.285	0.011
Na	0.140	0.712	0.047
P	5.500	0.000	0.997
Pb	3.965	0.062	0.135
S	18.920	0.000	0.485
Si	7.590	0.013	0.258
Sr	16.920	0.001	0.456
Ti	840.55	0.000	0.978
Zn	0.025	0.740	0.049

Table 4: Statistical comparison of heavy metals in liver of *C. punctatus* and *O. aureus.*

Trace Metal	F value	p (0.05)	Adjusted R^2
Al	2.791	0.000	0.993
Ba	100.172	0.000	0.839
Ca	244.72	0.000	0.928
Cr	4.220	0.055	0.145
Cu	3.563	0.075	0.119
Fe	73.686	0.000	0.793
K	350.51	0.000	0.948
Mg	40.243	0.000	0.674
Mn	79.473	0.000	0.805
Na	11.964	0.003	0.366
P	0.328	0.574	0.037
Pb	0	0	0
S	7.785	0.012	0.263
Si	9.509	0.006	0.309
Sr	173.126	0.000	0.901
Ti	1.122	0.304	0.006
Zn	0.378	0.546	0.034

Table 5: Statistical comparison of heavy metals in muscle of *C. punctatus* and *O. aureus.*

Trace Metal	F value	p (0.05)	Adjusted R^2
Al	951.305	0.000	0.981
Ba	47.117	0.000	0.708
Ca	7.227	0.000	1.000
Cr	0.139	0.713	0.047
Cu	0.214	0.649	0.043
Fe	3.481	0.078	0.116
K	62.0.63	0.000	0.763
Mg	13.623	0.002	0.399
Mn	0.484	0.495	0.028
Na	12.567	0.002	0.378
P	2.000	0.000	0.999
Pb	0.00	0.000	0.000
S	482.631	0.000	0.962
Si	0.001	0.976	0.056
Sr	5.724	0.000	0.997
Ti	0.269	0.610	0.040
Zn	3.465	0.079	0.115

Table 6: Human health risk assessment.

Metals	***C. punctatus***				***O. aureus***			
	EDI (Child)	**THQ (Child)**	**EDI (Adult)**	**THQ (Adult)**	**EDI (Child)**	**THQ (Child)**	**EDI (Adult)**	**THQ (Adult)**
Al	27.2	0.003	2.364	0.04	3.71	0.01	0.323	0.01
Ba	0.016	0.03	0.001	0.98	0.133	0.004	0.115	0.003
Ca	53.083	0.123	4.615	0.25	690	0.09	59.98	0.04
Cr	0.066	0.000	0.005	1.73	0.05	0.000	0.004	1.304
Cu	0.1	0.03	0.008	0.02	0.1	0.05	0.008	0.023
Fe	1.7	0.001	0.14	0.000	0.95	0.000	0.08	5.78
K	24.9	0.07	2.16	0.10	13.11	0.34	1.14	0.34
Mg	7.43	0.176	0.64	0.34	13.34	0.10	1.15	0.01
Mn	0.06	9.33	0.005	8.11	0.16	2.33	0.01	2.02
Na	23.18	0.012	2.01	0.20	29.36	0.030	2.55	0.20
P	42.71	0.014	37.21	0.41	403.33	0.45	351.34	0.04
Pb	0	0	0	0	0	0	0	0
S	100.2	0.002	8.71	0.32	47.91	0.32	4.16	0.45
Si	1.46	0.01	0.12	0.12	1.48	0.02	0.12	0.03
Sr	0.13	0.98	0.01	0.00	3.3	0.07	0.28	0.02
Ti	0.35	0.004	0.03	0.32	0.26	0.05	0.02	0.12
Zn	0.33	0.02	0.02	0.12	0.65	0.23	0.05	0.30

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