"Biochemical and Histological effects of heavy metals on fish from River Jehlum, Pakistan"

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HIGHLIGHTS

1. Collection of adult samples of *Labeo rohita*, *Catla catla*, *Ctenopharyngodon idella*, *Cirrhinus mirgala*, *Hypophthalymichthys molitrix*, *Oreochromis niloticus*, *Cyprinus carpio*, *Wallago attu* and *Sperata sarwari* from head Trimmu, river Jehlum, Pakistan
2. Quantification (in ppb) of Arsenic (As), Cadmium (Cd), Thallium (Ti), Lead (Pb) and Chromium (Cr) from muscles, kidney and liver of fish by wet digestion method, followed by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)
3. Assessessment of bioaccumulation of heavy metals in hepatic, renal and muscular tissues of fish samples
4. Proximate analysis w.r.t. body weight, edible portion and length of collected fish samples

ABSTRACT

Heavy metal contamination and different elements in trace amount are serious concern to global ecosystem, particularly human health, not only due to their toxic and mobile features, but because they also persist in the environment as being non-degradable. The aim of current study is more precisely to find out heavy metal quantity (ppb) by wet digestion, followed by Inductively Coupled Plasma Mass Spectrophotometry (ICP-MS) and their qualitative and quantitative effects on fish edible portion with respect to length and weight of fish, histopathology, biochemical profiling of liver, kidney and muscles of different fish of head Trimmu, river Jhelum, Pakistan. Statistically analyzed results showed that heavy metal content in kidney, liver and muscles of *Labeo rohita*, *Catla catla*, *Ctenopharyngodon idella*, *Cirrhinus mirgala*, *Hypophthalymichthys molitrix*, *Oreochromis niloticus*, *Cyprinus carpio*, *Wallago attu* and *Sperata sarwari* were in broad range (1.09– 4.7 ppb for arsenic, 0.01-0.48 ppb for cadmium, 0.05-0.32 ppb for thallium, 0.04-4.68 ppb for lead and 0.01-0.15 ppb for chromium), with broad range of edible portion (0.6- 1.125 Kg), which depended upon moisture (2.5 ± 0.8^{b}- 80.7 ± 4.40^{b} %), fat (0.63 ± 0.2^{d}-73.09 ± 2.14^{d} %), protein (1.64±0.83b- 58.35 ± 10.49^{c} %) and ash content (0.59 ± 0.17^{a}- 23.24 ± 1.13^{b} %) in fish muscles due to broad range of weight and length of each fish. Histopathological analysis showed that fish muscles have been affected more by all heavy metals, followed by the liver but maximum effect was due to arsenic. Since the available data for different heavy elements for fish is scanty, here an effort is made to present a precise data for the same. The analysis of by using the ICP-MS method shows the results are more sensitive, accurate, validated and economical and can be easily applied to raw materials and finished goods.

Keywords: *Head Trimmu, River Jelum Pakistan, Heavy metals, ICP-MS, Histopathology, Proximate and biochemical analysis*

1. INTRODUCTION

Heavy metal contamination is serious global concern particularly for human health due to their toxic and mobile features (Kamaruzaman et al., 2017, Razak et al., 2021) and their persistent behavior as being non-degradable (Selvi et al., 2019).With mounted industrialization and socio-economic development, metals of river systems has gained significant consideration due to prolonged persistence and lethal toxicity in aqueous environment (Ferati et al., 2015; Islam et al., 2015; Sun et al., 2019). River sediments are integral carrier and sink for all kind of metals, particularly heavy metals (Zhang et al., 2016; Guo et al., 2020) through runoff from agriculture, bedrock weathering , sewage treatment and atmospheric deposition(Li et al., 2020; Guo et al., 2020).Change in environmental parameters (e.g., redox potential, pH, bio- turbation, organic matter, and other conditions)discharge heavy metals from river sediments into larger water bodies (Albukhari et al., 2019); which affect environmental health and can be fatal for biotic factors (Raut et al., 2017; Ali et al., 2019; Xu et al., 2017 and 2018). Knowledge of metals in sediments is essential for the evaluation of aquatic environmental safety. Globally research has been conducted for understanding the prevalence, transport and deposition of heavy metals in river sediments (Ahmedet al., 2015; Aljahdali et al., 2020; Ma et al., 2016; Maharana et al., 2018; Nawab et al., 2018).Specific Tools have been devised to recognize the possible hazardous ecological threats due to heavy metals existence in river sediments (Islam et al., 2018), which provide solid scientific grounds for ecological management of metals in trace amounts even (Vickers, N. J., 2017; Jia et al., 2018).

Metals are categorized as essential, non-essential and toxic elements. Essential elements impose considerable beneficial effects on body metabolism, like zinc (Zn), copper (Cu), and iron (Fe) whereas ignorance about effect of non-essential ones like manganese (Mn), nickel (Ni) and vanadium (V). High concentration of both essential and non-essential elements can leave harsh impact(Uddin et al. 2020).Toxic elements like lead (Pb), cadmium (Cd), and chromium (Cr) generate toxicity on living ones and have no role in metabolism(Rubio-Iglesias et al. 2020).Human metals exposure is significantly via food consumption (Baharum et al. 2020).Metals intake via food chain or dietary intake is significantly addressable as it may become hazardous for human health by consuming contaminated fish with lower metal load even (Wang et al. 2021; Baki et al. 2018; Balali-Mood et al. 2021).

Fish, an integral human diet serves as source of nutrients like vitamins, proteins and unsaturated fatty acids(Islam et al., 2018), yet fish can pose potential risk if contaminated one (Duncan et al. 2018) as fish can easily be affected by the prevailing contaminants in localized habitat like water, sediments and diet (Islam et al. 2015; Carvalho et al. 2019).Metals, primarily accumulative in fish are greatly toxic and non-biodegradable, even if they are present in trace amounts (Mezzaroba et al. 2019).Heavy metal exposure have several implications for aquatic organisms such as crustaceans and fish (Majid et al. , 2018; Marin et al., 2022; Masindi et al., 2018; Maulu et al., 2021). Study by Masindi et al. (2018) showed decreased of heart rate, delayed hatching, and morphological change towards zebrafish during the exposure of nickel at 0.025 mg L^{-1}. Moreover, cadmium and lead caused several negative effects towards *Daphnia magna* such as brood delays and low performance in reproduction with the effect concentration (EC_{50}) of 21.02 µg L^{-1} and 694.57 µg L^{-1}, respectively (Orr et al., 2017). Adverse effects of heavy metal are not limited to aquatic organisms but also contribute to numerous diseases in human health (World Health Organization et al., 2017) as food chain is one of the main pathways for human exposure to heavy metals because they can accumulate and magnify in parts of the food chain such as water, sediment, zooplankton, and fish (Achary et al., 2017). Even though several metals such as iron and zinc are vital to the human body for cell growth and proliferation, irregular accumulation and successive excess of their intake can cause oxidative stress damage that leads to cancer (Wang et al., 2021). Arsenic can trigger lung and skin cancer even at the low exposure level of 8.1 µg L^{-1}, while cadmium is associated with ovarian and breast cancer through a dietary intake of more than 10 µg day^{-1} (Jarapala et al., 2014). A blood lead level of more than 70 µg L^{-1} can cause headaches, hypertension, fatigue, and loss of appetite. Whereas chronic exposure to lead can result in weight loss, birth defects, brain damage, and even death (Jan et al., 2015). Moreover, impaired cognitive development, hyperactive behaviors, and decreased intelligence quotient (IQ) points can be associated with manganese chronic exposure in water supplies up to 2.0 mg L^{-1} (Shomar et al. , , 2015). Gastrointestinal symptoms such as diarrhea, abdominal pain, nausea, and vomiting were reported at copper levels of 3 mg L^{-1} in drinking water (Taylor et al., 2020). Subsequently, aluminum adverse effects are linked with neurological diseases, Alzheimer's disease, and immunological systems breakdown at concentrations of 100 µg L^{-1} or greater, thus making the exposed groups more susceptible to other critical infections such as HIV (JRC, 2013, Shekhawat et al., 2015).

2. MATERIALS AND METHODS

2.1. Site description

Nine fish had been collected from Head Trimmu, located along the sides of river Jehlum (Fig. 1). River Jehlum is the most western and largest of all five rivers of Punjab, which passes through Jhelum district, originates from Verinag and flows through Indian-administered territory of Jammu and Kashmir, to the Pakistani-administered territory of Azad Kashmir, and then into Pakistani Punjab. Its total length is about 725 kilometers, with an average depth of 7,000 feet and an area of 34775 Km^2. It is located between 32° 56' 25.9728" N and between 73° 43' 39.4716" E, downstream of the Verinag River. The drainage basin of river is about 33,700 km^2. Water control structures being built on River Jhelum are Mangla Dam, Rasool Barrage/ Head Rasool, Trimmu Barrage/ Head Trimmu and Victoria Bridge/ Haranpur Bridge. Tourist activities and fishing by local fishermen are significant part of the economy for the local coastal inhabitants. It is a major tourist attraction because of its beautiful circular shape and forts, which adds its own stress on the ecosystem. Inputs from the river mouth to the Arabian sea are the routes of urban waste to this coastal environment.

Fig 1 Map showing head Trimmu on river Jehlum, Pakistan. Blue line shows river Jehlum water course

2.2. Field sampling

Twelve mature samples each of *Labeo rohita*, *Cattla Cattla*, *Ctenopharyngodon idella*, *Cirrhinus mirgala*, *Hypophthalymichthys molitrix*, *Oreochromis niloticus*, *Cyprinus carpio*, *Wallago attu* and *Sperata sarwari*), were collected from sampling site during two seasons summer (June) and winter (December) by professional fishermen using a multifilament, nylon gill net of 10 m length and 1.6 m height, but with meshes varying from 15 to 110 mm, knot to knot (Khan et al. 2017) and trawl from inside the river Jehlum during 2019- 2021, and identified upto species level using appropriate identification keys of Mirza and Sharif (1996) and Talwar and Jhingran (1991). Samples were washed with clean water at the point of collection, separated by species, placed on ice, brought to the laboratory on the same day and then frozen at -20 °C until dissection.

2.3. Biota

Frozen fish samples were thawed at room temperature and dissected using stainless steel scalpels. One gram of accurately weighed liver, kidney and epaxial muscle on the dorsal surface of the fish had been dissected for analysis. Dissected samples were transferred to Teflon beaker for the accomplishment of wet digestion (Rajeshkumar, S., & Li, X. 2020).

2.4 Wet digestion

Kidney, liver and muscles of each fish had been treated by wet digestion method according to the protocol of Bonsignore et al. (2018) respectively. Flasks has been prepared by washing all glass wares with soap and tap water, soaked in 10% nitric acid solution for 12 hours, followed by washing with deionized water and then dried them in hot air oven for 90 minutes moderate-temperature (not higher than 150 °C). In 2 cleaned and dried flasks (as "Sample 1 and as "Blank"), sample had been homogenized by shaking. 2g sample will be transfer into a digestion flask label with sample ID, while flask labeled as blank was empty. Few glass beads had been added into both flasks as anti-bumping granules, followed by addition of 20ml of 65% HNO_3 and 10ml of 70% perchloric acid and left them in fume hood to digest sample at ambient temperature for 24 hours. When samples turned into brown red color due to HNO_3 fumes, contents had been Shaked to mix them. Then flask has been kept on heat mantle of the digester at 120ºC to remove brown red fumes completely after 90 minutes of heat digestion. Digestion had been completed when 5-10ml of digestive juice left in the flasks. Samples have been diluted by deionized water through whatman filter paper No. 1.0 to appropriate dilution to make the calibration curve.

2.5. Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

Chromium (Cr), Lead (Pb), Arsenic (As), Thallium (Ti) and Cadmium (Cd) in fresh water fish were analyzed by using ICP-MS (PerkinElmer Élan 9000-USA). For better operating conditions the ICP-MS was adjusted to nebulizer gas flow 0.91 L/min, radio frequency (RF) 1200 W, lens voltage 1.6 V, cool gas 13.0 L/min, and auxiliary gas 0.70 L/min [29]. CRM samples were procured from Pakistan Institute of Nuclear Science and Technology (PINSTECH), Islamabad, Pakistan and used for method standardization and validation. Standard graphs were drawn while recovery study was also done (Janadeleh et al., 2018).

2.5. Histopathology of liver, kidney and muscles of fish species

Small slice of kidney, liver and muscles were collected for histological examination and preserved in 20 % formalin. Quantitative and qualitative analysis of TS of liver, kidney and muscles of all fish were done under the microscope with power of 40X (Lipsy et al., 2021).

2.6 Biochemical analysis of fish samples

2.6.1 The weight, length and edible portion of fish

Weight (Kg) and edible portion (Kg) has been calculated by pre-calibrated weighing balance while length (ft) has been calculated by an ordinary scale.

2.6.2 Proximate analysis of fish samples

2.6.2.1 Protein estimation

The Biuret method modified was employed for total protein estimation (Datta et al., 2018). 25 mg of dried tissue has been homogenized in a hand homogenizer with 1ml of glass-distilled water, followed by the addition of 4.0 ml of Biuret reagent in two installments of 2 ml each and a cleaned tissue grinder before transferring the content to the centrifuge tube. After 30minutes, the sample was centrifuged at 400 rpm for 10 minutes, collected the supernatant, and measured the optical density via UV-Visible spectrophotometer at 540 nm, by keeping the mixture of water and Biuret reagent as blank and Bovine Serum Albumin (BSA) as standard protein. The percentage of protein was calculated by using the following formula;

$$\text{Amount of protein (\%)} = \frac{\text{Standard value} \times \text{optical density}}{\text{Weight of the muscle tissue}} \times 100$$

2.6.2.2 Fat estimation

Chloroform -methanol extraction procedure of Oliva et al., (2003) was employed for lipid extraction from the muscle tissue. 400 mg of fine muscle powder has been mixed with 5,0 ml of chloroform -methanol (2:1) mixture by covering the test tube with aluminum foil and allowing it to stand for overnight digestion, followed by the filtration of the mixture in a pre-weighed 10 ml dried beaker. Difference between the weight of the empty beaker and beaker with residue gave the actual weight of the lipid in the muscle tissue. The percentage of lipid was calculated by using the formula;

$$\text{Amount of lipid (\%)} = \frac{\text{weight of the beaker with lipid} \times \text{weight of empty beaker}}{\text{Weight of the muscle tissue}} \times 100$$

2.6.2.3 Moisture estimation

The moisture content was estimated by subtracting the dry weight (dried in a hot air oven) of the muscle tissue from the known wet weight of the muscle tissue as follows (Haap et al., 2016);

$$\text{Amount of moisture (\%)} = \frac{\text{Dry weight of muscle tissue}}{\text{Wet weight of muscle tissue}} \times 100$$

2.6.2.4 Ash estimation

Ash was estimated by following the modified protocol of Achary et al., (2017) incinerating the preweighed test material in a muffle furnace at 560°C for 5 hours. The residue has been weighed, followed by the estimation of ash content.

2.6 Statistical analysis

In the present study, correlation analysis data were generated separately to compare the mean concentrations of trace amounts of heavy metals between different 09 species of fish, which are calculated at $p < 0.05$) for different fish species through GraphPad Prism 8.0, while visualization is also done by MS Excel too.

3. RESULTS

3.1. Metal Bioaccumulation in Tissues

Arsenic was present in liver and muscles of *W. attu* (1.32 and 4.5 ppb) and kidneys of *S. sarwari* (1.86 ppb) while it was absent in the tissues of rest of fish samples. Cadmium had been detected in the kidneys of *L. .rohita* (0.01 ppb) while it has not been detected in any other fish sample. Thallium toxicity has not been detected in the liver of *H. molitrix* only while it was present in liver, kidney and muscles of each fish species (0- 0.30 ppb). Lead toxicity has been detected in one or other or all tissues of fish species of head Trimmu from narrow to broad range (0- 1.22 ppb) except kidneys and liver of *C.Cattla* and *C. idella*, kidneys and muscles of *S. sarwari*, liver of *C. carpio* and muscles of *H. molitrix* and *C.Cattla*. Chromium traces has not been detected in liver of *H. molitrix* and kidneys of *S. sarwari* while it has been detected in all other fish samples (0.02-0.05 ppb) (Figure 1).

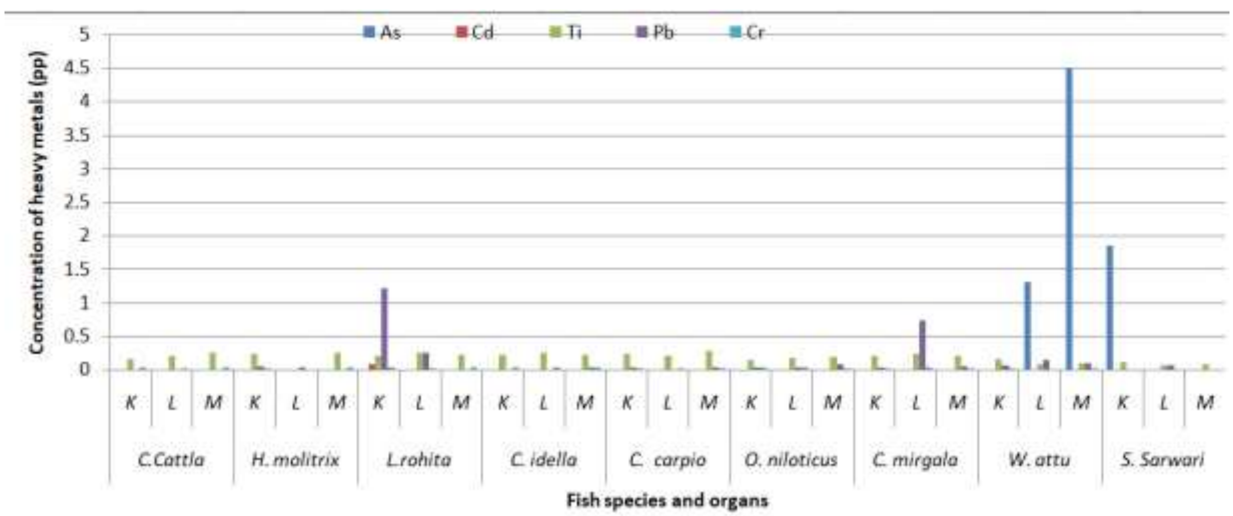


Figure 1Heavy metal content (ppb)in different organs of fish of river Jhelum

K= Kidney, L= Liver, M= Muscle, As= Arsenic, Cd= Cadmium, Cr= Chromium, Ti= Thallium, Pb= Lead

3.2 Histopathology of fish tissues

3.2.1 Histopathology of *C.Cattla*

Histological examination of its liver revealed slight degeneration of hepatocytes and slight vacuolar degeneration, with slight necrotic changes but atypia or malignancy was absent (Figure 2 A). Histological examination of kidney of this fish revealed scattered inflammatory cells in the interstitium. Some of the tubules had eosinophilic hyaline casts, with evident focal degeneration of glomeruli. Renal tissue necrosis was absent but tubular degeneration, fibrosis, atypia or malignant changes were present (Figure 2 B). The pathological findings in fish showed broken of myofibrils, Splitting of muscle fibers, disintegrated myotomes and vacuolation within the myotomes (Figure 2 C).

3.2.2 Histopathology of *H. molitrix*

Histological examination its liver had normal architecture of liver tissues with dilatation of central veins. There was associated hyperplasia of tubules with peritubular cuffing of lymphocytes. Hepatic degeneration, inflammation, fibrosis, necrosis, atypia or malignant changes were absent (Figure 3 A) while histological examination of kidney revealed minor hydrophobic degeneration of tubules. Overall renal capsule, nephrons and tubules were looking normal while renal tissue inflammation, necrosis, fibrosis, atypia or malignant was also absent (Figure 3B). The histology of muscle tissue showed various degrees of deterioration when compared to control. The deterioration was very much reduced in the treated sago effluent when compared to the untreated sago effluent (Figure 3C).

3.2.3 Histopathology of *L.rohita*

Histological examination of liver revealed mild to moderate hydropic degeneration of hepatocytes and slight congestion of hepatic sinusoids. Dilatation of central veins was evident but inflammation, fibrosis, necrosis, atypia or malignancy was absent (Figure 4 A). Histopathological examination of kidney revealed mild to moderate renal tubular degeneration, with mononuclear cells infiltration. In intratubular areas, the tissue showed focal chronic inflammation but renal tissue necrosis, fibrosis, atypia or malignant had not be seen (Figure 4 B). Drastic changes in the fatty acids profile and severe histological abnormalities viz. shortening of muscle bundles, edema, hyper-vacuolization, elongation of muscle bundles, gap formation in myofibrils, degenerated myotomes, hemorrhage, inter-myofibrillar space, necrosis, were also recorded in muscular tissue of exposed fingerlings. The intensity of muscular damage in *L.rohita* was found to increase with increase in duration of exposure (Figure 4 C).

3.2.4 Histopathology of *C. idella*

Histology of liver revealed mild to moderate hydropic degeneration of hepatocytes and slight congestion of hepatic sinusoids, central vein; epithelial layer; hepatocytes; lipid type vacuolization; ruptured central vein; lymphocyte infiltration (Figure 5 A). Histology of kidney revealed mild to moderate renal tubular degeneration, with no gross changes in renal capsule and nephron. Renal tissue inflammation, necrosis, fibrosis, atypia or malignant had not be seen (Figure 5 B). Abnormalities such as single and double micronuclei, deformed nucleus, nuclear shift, irregular nucleus, deformed cells, microcytes, and vacuolated and swollen cells were observed in its muscles. Inflammation and necrosis of muscle fibers, degeneration of muscle fibers, edema of muscle bundles, zig-zag of muscle fibers, and lesions were observed in muscle tissues of fish exposed with different doses of these heavy metals (Figure 5 C).

3.2.5 Histopathology of *C. carpio*

Histology of liver of this fish has shown reduction in hepatic injury but still hepatic lesions and inflammation of cells had been seen (Figure 6 A). Histology of its kidney showed inflammation on cells and damage Bowmen's capsule, with podocytes and mesangium which leads to kidney damage (Figure 6 B). The photomicrograph of the muscle depicted the presence of normal myotomes with equally spaced muscle bundles. On exposure to sub-lethal concentration of lead, marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed. Sublethal concentration of cadmium (Figure 6 C) led to pronounced intramuscular oedema with minor dystrophic changes.

3.2.6 Histopathology of *O. niloticus*

Histology of its liver had hepatic lesion and pigmentation, with swelled and inflamed cellular wall but the liver damage was minimum (Figure 7 A). Histology of kidney showed that vascular poles were not in normal in structure and had comparatively less erythrocytes in glomerular capillaries. Their kidneys have little convoluted proximal tubules and normal juxtaglomerular cells. Karyolysis has also been observed in cells and tissues that lead to severe kidney damage (Figure 7 B). In the muscle there was mild lesion, necrosis, inclusion bodies, inflammation and cellular degenerations (Figure 7 C)

3.2.7 Histopathology of *C. mirgala*

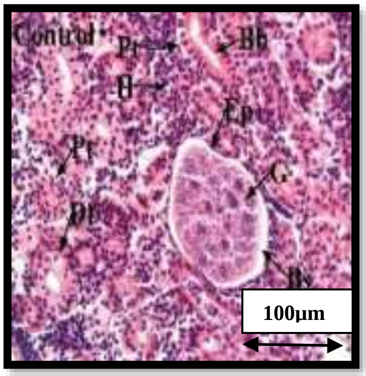
Histology of its liver has shown less cell inflammation and hepatic lesions (Figure 8 A), while that of kidney revealed karyolysis in renal cells and also showed mild kidney damage. Macula densa and afferent arteriole were properly packed and structurally stabled. Space for bowmen's capsule had been reduced and podocytes were structurally in stabled form (Figure 8 B). Muscle sections of *C. mrigala* harvested from the polluted section of river demonstrated the necrosis, degeneration of muscle fibers, intra-fibular edema and release of the blood into the tissues due to the bursting of blocked of the blood vessels. Dermal layers showed degeneration of the collagen bundles those were found loose or collapsed in some regions. Photomicrography also revealed vacuolar degeneration in muscle tissues and atrophy of muscle bundles. Intra fibular edema and splitting of muscle fibers were also seen along with bioaccumulation of toxicants (Figure 8 C)

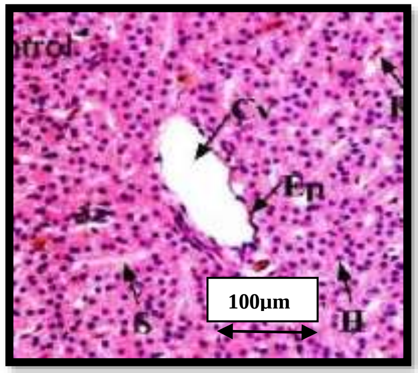
3.2.8 Histopathology of *W. attu*

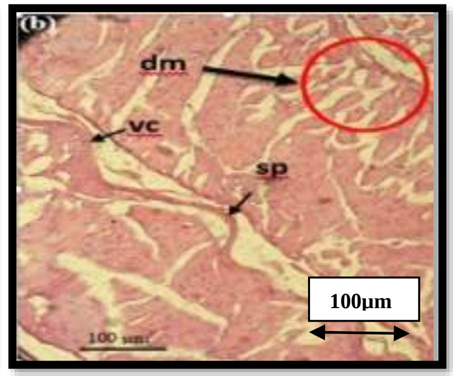
Histology of its liver has shown less damage because swelling of cellular wall and pigmentation was less (Figure 9 A). Histology of kidney showed that proximal tubules were mild convoluted that showed less kidney damage. Juxtaglomerular cells and podocytes were in slightly perfect form, but urinary pole showed mild kidney damage (Figure 9 B). *W. attu* showed maximum incidence of alterations in muscles with highest histopathological alteration index related to environmental degradation.(Figure 9 C).

3.2.9 Histopathology of *S. sarwari*

Histology of its liver showed high level of pigmentation in hepatocytes (Figure 10 A), with lesions and pathological changes of renal tubules. The urinary poles were normal and in proper shape. Karyolysis had been observed with normal nuclei, capsule and glomeruli (Figure 10 B). Muscles of this fish had shown maximum alterations in striated muscles which present heavy metal toxicity in fish muscles (Figure 10 C)







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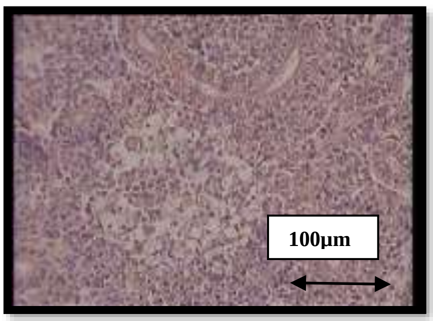

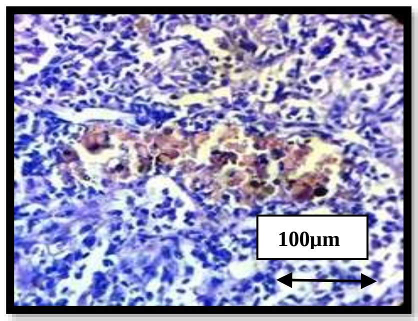

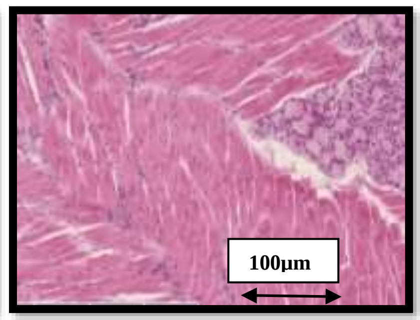

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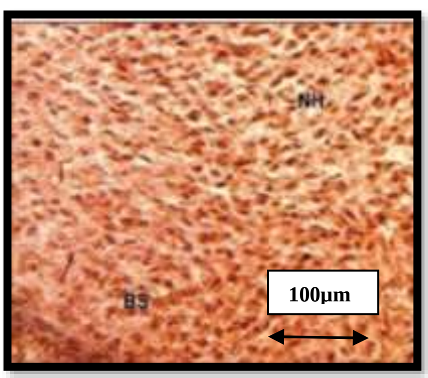

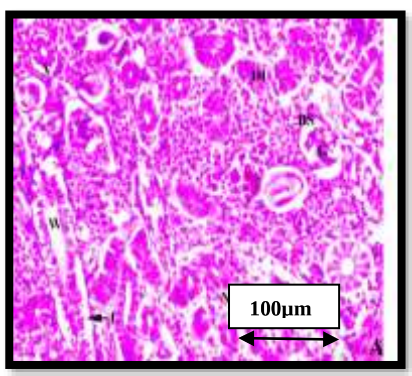

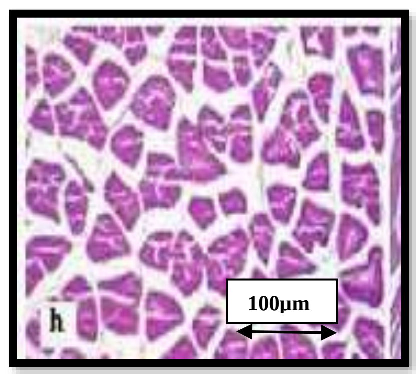

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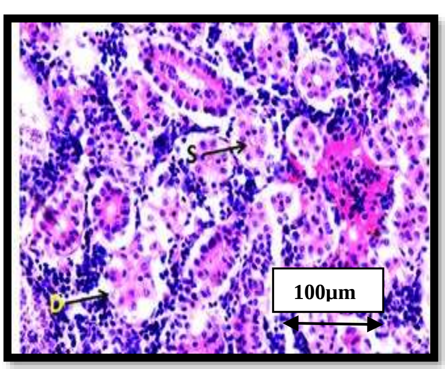

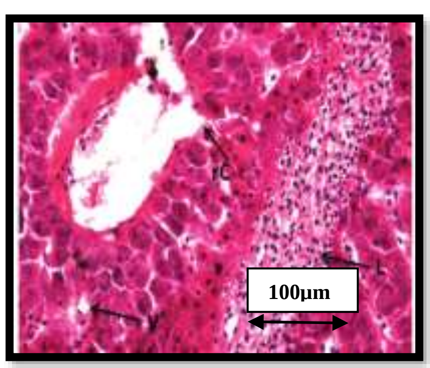

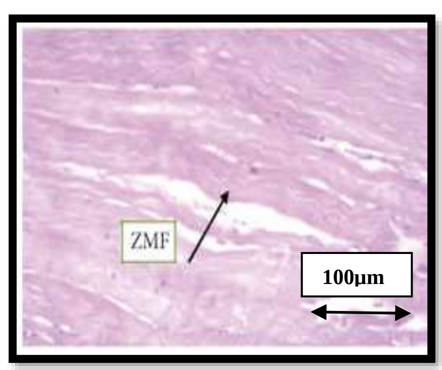

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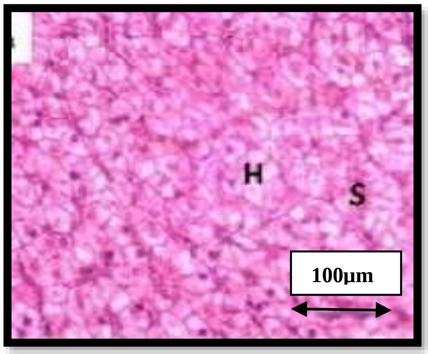

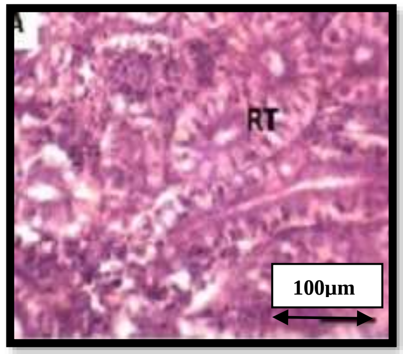

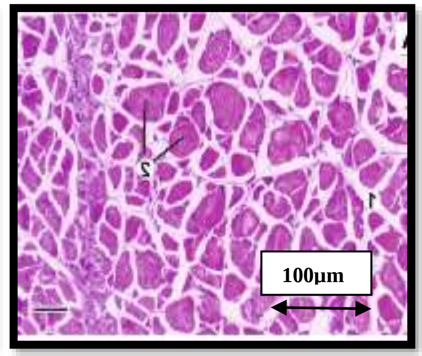

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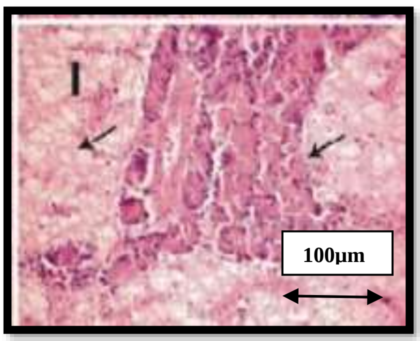
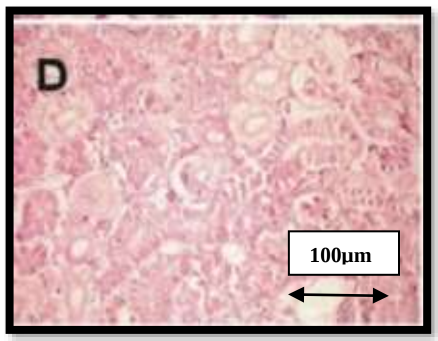
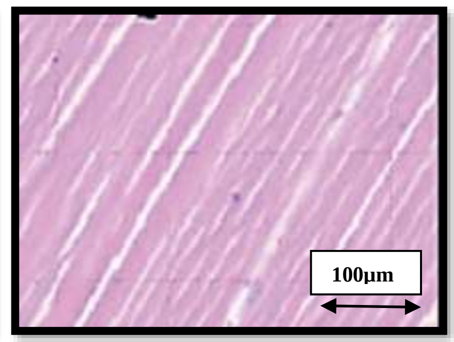
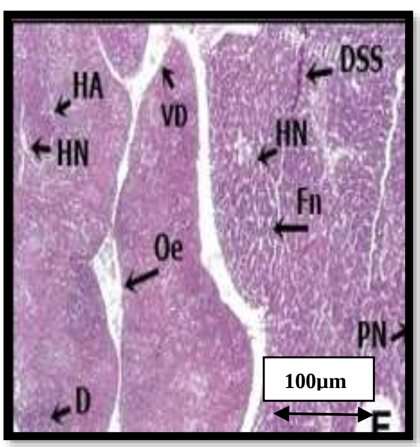
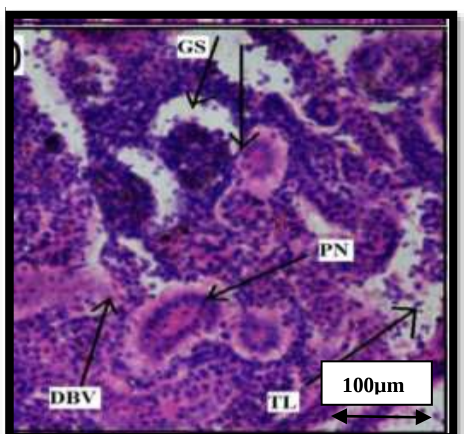


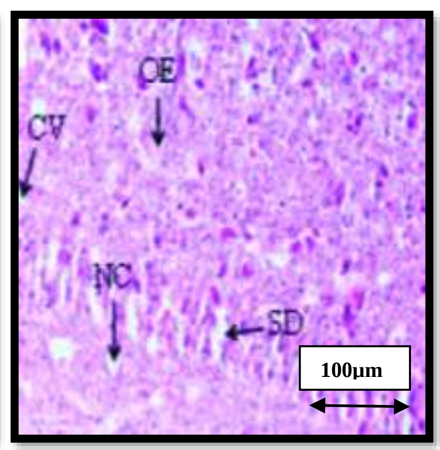




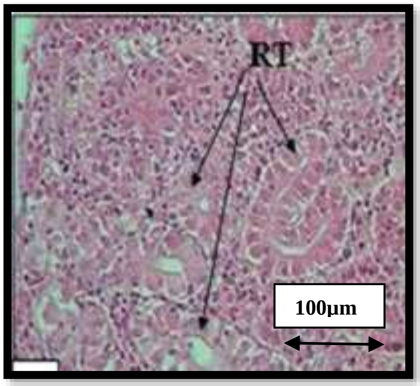
7. A **7. B** **7. C**



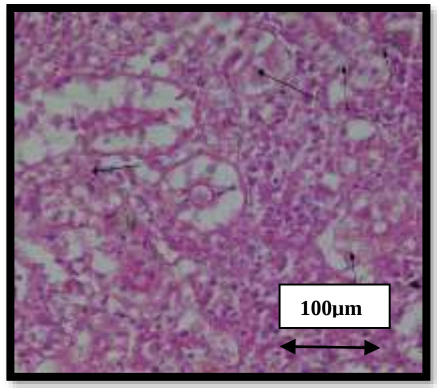




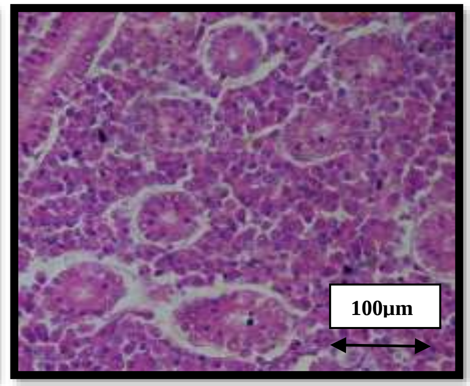
8. A **8. B** **8. C**



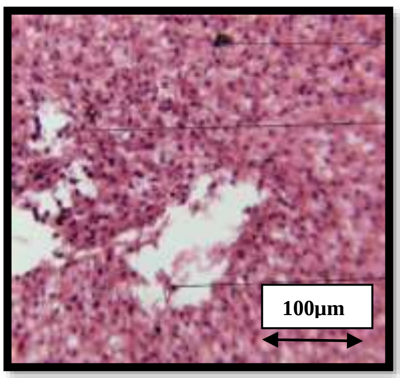
9. A



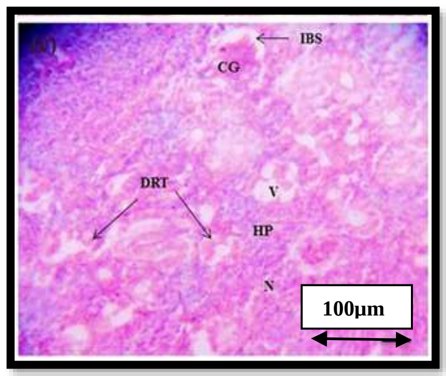
9. B



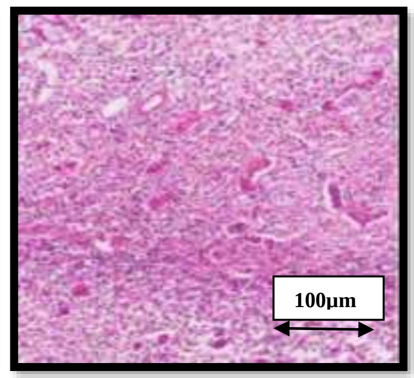
9. C



10. A



10. B



10. C

Figure 2 to 10 Histopathology of liver (A), kidney (B) and muscles (C) of fish species from head Trimmu of river Jehlum

Figure 2= *C.Cattla*, **Figure 3=** *H. molitrix*, **Figure 4=** *L.rohita*, **Figure 5=** *C. idella*, **Figure 6=** *C. carpio*, **Figure 7=** *O. niloticus*, **Figure 8=** *C. mirgala*, **Figure 9=** *W. attu* , **Figure 10=** *S. sarwari*

Cytoplasmic vacuolation (CV), Necrosis (NC), Oedema (OE) and Sinusoid dilation (SD). Congestion of sinusoids (CSS), Hepatocyte atrophy (HA), Pyknotic nuclei (PN), Haemorrhage (H), Oedema (Oe), Dilation of sinusoids (DSS), Vascular dilation (VD), Cytoplasmic Vacuolization (CV), Partial degeneration of hepatocytes (PH), Fatty degeneration (D), Focal area of necrosis (Fn), Hepatic cells Inflammation and Structural alterations (SA) Glomerular shrinkage (GS), Increase space between glomerulus and Bowman's capsule (IS), Increase tubular lumen (TL), Pycnotic nuclei (PN), disorganized tubules (DT), Hydropic swelling (HS), Desquamation (D) and Damage blood vessels (DBV), G (glomeruli), BS (bowman's space), RT (renal tubule), IBS (increased Bowmans space), DG (damaged glomeruli), DRT (damaged renal tubule), N (necrosis), HRT (hypertrophied renal tubule), V (vacuolation), HP (hyperplasia), CRT (constricted renal tubule), CG (constricted glomeruli), GF (granuloma formation)

3.3 Biochemical Analysis of fish samples

3.3.1 Edible portion, weight and length of fish (Table 1)

Weight of all fish collected from head Trimmu was in the range of 0.7- 2.5 Kg, lengths of all fishes were in the range of 0.5-2.5 ft and edible portion of all samples was in the range of 0.6- 1.125 Kg, depending upon the weight and length of each fish (Table 1).

3.3.2Proximate Analysis of fish and recovery study for heavy metals

Amount of moisture, fat, protein and ash was found to be highest in *C. idella* (80.7^{b}±4.40, 63.89^{c}±10.14, 58.35^{d}±10.49 and 17.76^{b}±3.46 % respectively) while they were found to be least in *C.Cattla* (3.9 ^{a} ±0.3 % moisture), *O. niloticus* (5.75^{b}±0.31 % protein and 0.91^{a}±0.19 % ash) and *W. attu (*0.85^{d}±0.25 % fat) (Table 2). Moreover, recovered amount of heavy metal ranged from 81.0 % to 120.5 % for Cr, Pb, As, Cd and Ti (Table 3).

Table 1 Weight, Edible portion and length of fish collected from head Trimmu

Scientific name	Common name	Trophic levels	Edible portion/kg	Weight (Kg) (min.–max.)	Length (ft) (min.–max.)
C. cattla	Thaila	Planktivores	1.125	2.5	**2**
H. molitrix	Silver Carp	Planktivores/ detritivores/omnivore	1.125	2.5	1.5
L. rohita	Rahu	herbivore/Planktivores	2.25	5.0	2.0
C. idella	Grass Carp	Herbivore	1.35	3.0	1.0
C. carpio	Common carp	Omnivore	0.6	1.5	0.75
O. niloticus	Talapia	Omnivore	o.315	0.7	0.5
C. mirgala	Mohri/Morakhi	herbivore/detritivores	0.6	1.5	1.5
W. attu	Malhi	Carnivore	0.9	2.0	1.5
S. sarwari	Singhari	Carnivore	0.45	1.0	6.0

Table 2 Proximate composition in fish muscles (%)

Scientific name	Moisture	Fat	Protein	Ash
C.Cattla	$3.9^{a} \pm 0.3$	$5.2^{c} \pm 1.3$	$16.22^{a} \pm 1.5$a	$3.1^{b} \pm 1.1$
H. molitrix	$4.4^{b} \pm 1.1$	$6.9^{b} \pm 1.2$	$17.34^{a} \pm 1.9$	$3.5^{a} \pm 1.3$
L.rohita	$7.7^{a} \pm 0.20$	$0.98^{d} \pm 0.20$	$18.83^{b} \pm 0.29$	$2.30^{b} \pm 0.09$
C. idella	$80.7^{b} \pm 4.40$	$63.89^{c} \pm 10.14$	$58.35^{d} \pm 10.49$	$17.76^{b} \pm 3.46$
C. carpio	$7.84^{c} \pm 1.3$	$2.1^{a} \pm 0.23$	$8.34^{b} \pm 1.29$	$1.1^{a} \pm 0.61$
O. niloticus	$8.11^{b} \pm 1.51$	$1.53^{b} \pm 1.23$	$5.75^{b} \pm 0.31$	$0.91^{a} \pm 0.19$
C. mirgala	$3.1^{b} \pm 0.4$	$8.8^{a} \pm 1.4$	$17.9^{b} \pm 1.21$	$3.3^{a} \pm 0.28$
W. attu	$70.88^{a} \pm 0.43$	$0.85^{d} \pm 0.25$	$19.21^{c} \pm 1.23$	$2.90^{b} \pm 0.14$
S. sarwari	$50.99^{c} \pm 0.45$	$19.15^{a} \pm 0.98$	$21.38^{a} \pm 1.54$	$1.45^{b} \pm 0.06$

Values are given as mean ± S.D. from triplicate determinations; Different superscripts in the same column indicate significant differences ($p < 0.05$).

Table 3 Recovery study using certified reference material (CRM) for heavy metals

Elements	Analyzed value (mg/kg dry weight)	Certified value (mg/kg dry weight)	% of recovery
Chromium	201	177	87.9
Lead	101	95.4	94.4
Arsenic	4.31	3.51	81.0
Cadmium	104	100	96.2
Thallium	16.6	20	120.5

DISCUSSION

Fish is one of the main sources of easily digestible protein rich in essential amino acids, fats, macro- and trace elements, and fat-soluble vitamins. Fish is a food rich in valuable long chain polyunsaturated omega-3 fatty acids. The appropriate quantities of n-3 PUFA, such as eicosapentaenoic (EPA, C20:5 n-3), docosahexaenoic (DHA, C22:6 n-3) and ocosapentaenoic acid (DPA, C22:5 n-3), prevent or reduce the risk of cancer, cardiovascular diseases and neurological disorders. Scientific studies have confirmed that PUFAS play an important role in the growth of the fetus and the development of cognitive functions in children. However, fish also have the ability to accumulate trace elements, heavy metals, pesticide residues, and persistent organic pollutants in their tissues, including polychlorinated biphenyls (PCBs). The organic pollutants, next to heavy metals, can be harmful to the aquatic ecosystem and humans by the consumption of fish (Luczynska et al., 2022).

Fish accumulate substantial amounts of metals in their tissues, especially in the muscles. Many factors influence the metal contents in fish tissues, such as environmental quality, season, fish species, stage, and age of maturity (Luczynska J. and Paszczyk B. 2019). The specific diet of age groups of a given species and the bio-indicator capacities of different age groups are also important. Fish samples are considered as one of the most indicative factors in freshwater systems for the estimation of the heavy metal pollution potential (Nyeste et al., 2019).

Consumption of fish worldwide has increased speedily in recent years, particularly with the awareness of its nutritional and therapeutic benefits. In addition to being an important source of protein, fish are enriched with essential minerals, vitamins, and unsaturated fatty acids. The American Heart Association recommended consumption of fish at least twice per week to reach the daily intake of omega-3 fatty acids. However, fish normally accumulate heavy metals from food, water, and sediments, and this is a good indicator of heavy metal contamination in water. The presence of toxic heavy metals in fish can invalidate their beneficial effects. Several unfavorable effects of heavy metals on human health have been known for a long time. This includes serious threats like renal failure, liver damage, cardiovascular diseases, and even death. Thus, many local and international monitoring programs have been established to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem. According to the literatures, metal bioaccumulation by fish and subsequent distribution in organs is greatly interspecific. In addition, many factors can influence metal uptake like sex, age, size, reproductive cycle, swimming pattern, feeding behavior, and geographical location (Bawuro et al., 2018).

Current study showed that arsenic toxicity has been detected in all organs of *W.attu* and *S.sarwari* from head Trimmu. At this sites the concentration of arsenic observed higher when compared to standard guide lines value 0.05mg/L (Hafiza et al., 2017) or 10 µg/L (Azmat et al., 2016) of WHO or Pakistan's safe limit of 50 mg/Lin 65% sources may pose serious cancer risks for 2 to 5 persons (maximum 12 persons) per 10,000 population. Naturally, arsenic is present in metalloid form. Arsenic accumulation leads to discoloration of the skin and causes cancer (RizwanUllah et al., 2018). Although geologically released As is a major source of groundwater contamination (Ayele et al., 1993), many anthropogenic activities are also thought to be responsible for contamination of groundwater and surface water with As (SDWF.,2003). It is a ubiquitous toxic element in the geothermal system (Farooq et al., 2012) and present in about > 200 mineral forms, but the most common minerals are arsenical pyrite (FeAsS), orpiment (As2S3) and realgar (AsS). The geochemical alteration in underground sediments could trigger As release in aquifers. For example, water-rock interaction, sorption/desorption, oxidative/reductive dissolution processes of As-bearing FeAsS (Nickson et al., 2005) and/or Fe oxides can result in contamination of groundwater systems (Cvjetko et al., 2010). The high as levels in groundwater might also be attributed to oxidative desorption with a rise in the evaporative concentration process with other physicochemical parameters (Bagchi et al., 2002).

Trace amount of cadmium has been detected in different organs of different fish from head Trimmu. The Cd concentration at this site was observed higher when compared to WHO guidelines (0.003mg/L) (Hafiza et al., 2022). Through paint, glass enamel, pigments, and deterioration of the galvanized pipes, cadmium is added to the surface waters (Moore JW and Ramamoorthy S., 1984). Few cases of Cd poisoning are reported in humans by ingestion of contaminated fishes. Its toxicity is low in the case of plants than Cu. Cd is toxic to invertebrates and fishes (Sindhu, PS., 2002). Cd effects on human health by kidney and liver damage, renal malfunction, and gastrointestinal damage (Ashraf et al., 1991).

Thallium and lead toxicity has been detected in all fish samples, but was less prominent in fish samples of head Trimmu. At all sites the lead concentration was higher when compared with the standard value of 0.01mg/L suggested by WHO (Hifza et al., 2022). The most ancient metal known to man is lead and is released into surface water by pipes, building materials, gasoline and paints etc. It is a familiar as poisonous metal (Natasha et al., 2021). It affects human health like kidney damage, digestive disturbance, brain and nerve damage, blood disorders, and hypertension (Palaniveloo et al., 2020). High level of lead has been mainly assigned to human activities. Surface water quality is the main issue particularly in regions where it is used for drinking purposes (Amen et al., 2020). While thallium is a waste product of coal combustion and the manufacturing of cement and this industry is widespread in the premises of river Jehlum. Industrial use of thallium is very extensive, mostly in specialized electronic equipment. Most gamma radiation detection equipment such as scintilla meter and infrared radiation detection and transmission equipment contain thallium as an activator. Thallium-barium-calcium-copper oxide high-temperature superconductors (HTS) are used in filters for wireless communications. This use in particular threatens to increase the demand for thallium because at the moment, thallium materials are superior and more cost-effective for these very specialized uses. Thallium-arsenic-selenium crystals are essential in filters for light diffraction in acousto-optic measuring devices. Thallium is used in an alloy with mercury to measure low temperatures. Other uses include an additive in glass to increase its refractive index and density, a catalyst or intermediate in the synthesis of organic compounds, and a component in high-density liquids for sink-fly oat separation of minerals. Man-made sources of thallium pollution are gaseous emissions from cement factories, coal burning power plants, and metal sewers. Leaching of thallium from ore processing operations is the major source of elevated thallium concentrations in water. Major industrial sources of elevated thallium include primary copper smelting, petroleum refining, primary nonferrous metals, blast furnaces, leather industry, and steelworks (Anawar et al., 2004). The maximum contaminant level (MCL) for thallium in drinking water, at which no known adverse effects on human health are anticipated, is 0.002 mg L^{-1} and According to US EPA, MCL is the lowest level at which water suppliers can reasonably be required to remove the contaminant of interest, should it occur in drinking water while level for a child exposed to thallium is 0.007 mg L^{-1} , generally accepted limit for thallium content in arable soil is 1 mg kg^{-1} and Worldwide limits for crops and land plants are 0.03 mg kg-1 to 0.3 mg kg^{-1} (dry mass) and 8.0 µg kg-1 to 1 mg kg-1 (dry mass), respectively (Hussain et al., 2020). Thallium salts are rapidly and nearly completely absorbed by virtually all routes, with gastrointestinal exposure being the most common route to produce toxicity. Thallium enters cells by a unique process governed by its similarity in charge and ionic radius to potassium. Although the exact mechanism of toxicity has not been established, thallium interferes with energy production at essential steps in glycolysis, the Krebs cycle, and oxidative phosphorylation. Additional effects include inhibition of sodium-potassium-adenosine triphosphatase and binding to sulfhydryl groups. The major manifestations of toxicity consist of a rapidly progressive, ascending, extremely painful sensory neuropathy and alopecia. Unlike exposure to most metal salts, gastrointestinal symptoms of thallium toxicity are relatively minor, and constipation is more characteristic than diarrhea. Many other findings such as autonomic neuropathy, cranial nerve abnormalities, altered mental status, and motor weakness; cardiac, hepatic, and renal effects are described, but are less specific. Thallium also crosses the placenta freely and produces abnormalities in animals as well as fetal demise, overt toxicity, and congenital abnormalities in humans. There are no controlled trials of treatment in thallium-poisoned patients (Kumar, A. and Singh, C.K., 2020).

Trace of chromium have been detected in all fishes' head Trimmu, which was above the limit suggested by WHO (0.05mg/L) (Hafiza et al., 2017). The higher concentration of Cr might be attributed to anthropogenic activities such as effluents from dyeing and tanning industries or naturally due to weathering of crustal materials (Natasha et al., 2020). Higher concentrations of chromium caused air passage and lung problems (Robert S. and Hoffman., 2003).

Pakistan is a developing country facing surface water pollution that may cause serious threats to aquatic life. Aquatic organisms quickly respond to minor changes in the environment that reflect the health status of a water ecosystem. Fish are an important and sensitive member of the aquatic food web. Most of their species are confined to live in microhabitats; however, when these habitats get contaminated, a variety of fish species either moves to less polluted areas or dies. Moreover, the presence of heavy metals may pose lethal or chronic effects on fish fauna. Toxic effects are increased when different metabolic activities fail to detoxify the metals in the body of an organism. Heavy metals can bio-accumulate in aquatic biota and bio-magnify in food chains. In aquatic organisms, metals can act as mutagenic compounds and can affect tissues at the organ, cellular, sub-cellular and molecular levels. Biochemical and physiological activities of both the blood and tissues of fish are affected by heavy metals (USGG Mineral Information., 2010; Cvjetko et al., 2010).

Conclusion

It is concluded that fish from Khushab river, Jehlum, head Rasool, and head Trimmu had more heavy metal toxicity as compared to fish of Mangla dam and this toxicity was more prominent in hepatic and muscular tissues of fish. Therefore, consumers should rely more on Mangla dam fish for food and pharmaceutical industry. Moreover, all those anthropogenic activities should be monitored by the environmental and health care department and strict measures should be taken by the local population as well for the reduction of heavy metal release in sampling sites of river Jehlum.

DISCLAIMER

The fished used for this research are commonly and predominantly used in our area of research and country. There is absolutely no conflict of interest between the authors and fishermen because we do not intend to use these fishes as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by any agency or company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

FUNDING STATEMENT

Work has been done without funding of any agency or department.

LIMITATION OF STUDY

These are:

- Data from other sites of river Jehlum, Pakistan has not been compared with current data.
- Different resources to reduce or eradicate the release of heavy metals in river Jehlum has not been mentioned.
- It is not mentioned that what are government initiatives to identify heavy metal toxicated fishes before their release in local market which could indirectly have strong impact on associated health risks.

FUTURE RECOMMENDAITON

The current work can be used for

- The comparative analysis of heavy metal contents and its impacts on fish tissues from different sites of river Jehlum, Pakistan.
- The usage of different resources and government initiatives to reduce or eradicate the release of heavy metals in river water.
- Further studies for the identification of changes at molecular level in fish tissues due to heavy metals, which could be used for the formulation of plant based anti-toxicants for not only fish population but also for other aquaculture populations in river water.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

Credit authorship contribution statement

NK performed the whole experiment in the lab, search the literature for this manuscript, provided English editing services, and wrote the first draft of this manuscript, AMK supervised the whole work, designed the project and given final approval to this manuscript. AA collected the literature and provided technical support and English editing services for this manuscript. NAA provided technical support for the quantification of heavy metals and drafted this part in manuscript. MAS supervised the project during quantification of heavy metals through ICP-MS, provided English editing services and given final approval to this manuscript. BR provided English editing services for this manuscript and technical support for the collection and identification of fish from different sites and drafted this part in manuscript. SA helped in the collection and identification of fish samples and RB provided her technical support for the compilation of this data and proof-read manuscript.

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