EFFECT OF HEAVY METALS BIOACCUMULATION ON THE VITAL ORGANS OF MAJOR CARPS (*LABEO ROHITA, CATLA CATLA AND CIRRHINUS MRIGALA)* IN CONFINED AND NATURAL WATER BODIES

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Requirement for the Degree of

MS Zoology

By

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No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the **'Faculty of Sciences**, The Superior University, Lahore in partial fulfillment of the requirements for the degree of Master of Philosophy in the field of **"Zoology"** in Faculty of Sciences at The Superior University, Lahore.

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DEDICATION

I want to sanctify this humble effort to

My Affectionate Parents

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In the name of **ALLAH**, the most Gracious, the most Merciful. I am extremely thankful for the **Almighty 'ALLAH'** Who is the entire source of knowledge and wisdom endowed to mankind, for providing me with the insight and vision to complete this endeavor. I offer humblest thanks and countless salutations to **HOLY PROPHET MUHAMMAD (PBUH),** who is forever, a torch of guidance for the entire humanity.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BDL	Below Detectable Limit
DO	Dissolve Oxygen
DMR	Duncan's Multiple Range
EC	Electrical Conductivity
EPA	Environmental Protection Agency
GLM	Generalized Linear Model
PH	Potential of Hydrogen
S.M.E.W.W.	Standard Methods for the Examination of Water and Wastewater
TDS	Total Dissolved Solids
YSI	Yellow Spring Instrument

ABSTRACT

The present study evaluates the impact of heavy metal bioaccumulation on vital organs in major carps (*Labeo rohita*, *Catla catla* and *Cirrhinus mrigala*) from confined and natural water bodies. For this purpose, fish and water samples were collected from three sites of Punjab: The Sulemanki Headwork's, Balloki Headwork's, and a controlled Fish Farm at UVAS Ravi campus. Atomic absorption spectrophotometry was used to estimate heavy metals in water and fish tissues (gills and liver) compared to EPA 2020 limits. To discover pathological alterations induced by heavy metal exposure to fish, gill and liver tissues were histopathologically examined. ANOVA and Duncan's Multiple Range Test were used to compare location and tissue metal accumulation. The present study revealed that heavy metal accumulation was significantly ($P \leq 0.05$) different between the three sites, where the Sulemanki Headwork's site was the most contaminated, especially in the liver tissues ($P \leq 0.05$). The concentration of heavy metals across all three sites revealed that chromium was the most prevalent, while cadmium exhibited the lowest concentration. The liver exhibited the highest accumulation of heavy metals ($P \leq 0.05$) followed by the gills, whereas the water samples showed the lowest concentration ($P \leq 0.05$) Histopathological studies revealed significant impairments in the gills ($P \leq 0.05$) and liver tissues ($P \leq 0.05$), with the presence of heavy metals indicating a strong link between contamination and tissue damage. This study observed that agricultural and industrial runoff significantly affects the aquatic ecosystems, especially in natural water bodies. The bioaccumulation of heavy metals in selected organs of the fish presents significant dangers to fish health and to the people consuming them.

Keywords: Carps, histopathology, heavy metals, aquatic pollution, gills, liver

CHAPTER 1

INTRODUCTION

Water bodies are very important in supply of ecological fragments and contributing to the support of diverse species. They act as a home to millions of species, for instance fish that are very critical for food and business to many people around the globe [1]. Water in Particular River, lakes and ponds provides a crucial habitat to support the fish production [2] Among these the major carps *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala* are important in terms of aquaculture and fisheries alike mainly due to uses in fisheries [3].

1.1. Major Carps

Among the major carps, *Labeo rohita* known as rohu, *Catla catla* known as Thaila and *Cirrhinus mrigala* commonly known as Mori are the three originating from South Asia. In recent years, these species have gained popularity in aquaculture due to their rapid growth rate, high nutritional value, and adaptability to favorable farming environments. *Labeo rohita is* widely favored by consumers because of its unique flavour and appealing texture, making it a preferred choice in the market. *Catla catla* is characterized by having a large head compared to its body weight and very fast growth while *Cirrhinus mrigala* on the other hand is characterized by adaptability to various conditions of the environment and high resistance to diseases [4]. These carps are very important for fisheries and are a key link in the food chain in aquatics, they also involve themselves in the cycling of nutrients and energy flow in their area [4].

1.2. Relevance of Studying Heavy Metal Bioaccumulation

Studying the bioaccumulation of heavy metals in fish is significant because these metals are poisonous to fish and other organisms as well. Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As) and many others are said to contaminate water through industrial effluents, agricultural run offs, and leaching from mining activities [5]. Aquatic organisms tend to absorb and retain heavy metals in their tissues, which can lead to toxic effects that hinder growth, disrupt reproductive processes, and increase mortality rates. This study aims to explore how

heavy metals accumulate in fish and assess the resulting biological impacts. The findings are intended to support efforts to control environmental pollution, protect aquatic biodiversity, and ensure the safety of fish for human consumption [5].

1.3. Heavy Metals in Aquatic Environments

1.3.1 Sources of Heavy Metal Pollution

Hg, Cd, Cr, Pb, Ni, Mn and Cu are named as heavy metals which are discharged into water from natural and anthropogenic sources. Heavy metal pollution in water sources primarily stems from human activities such as industrial discharge and agricultural runoff. Farming practices, particularly the use of metal-containing pesticides and fertilizers, contribute significantly through surface drainage [6]. While natural processes like volcanic eruptions and rock weathering can also release metals into water bodies, anthropogenic sources remain the major contributors. Littering and Discharging raw sewage increase the compound water pollution index and the pollution level respectively as highlighted by [7] .

1.3.2 Types of Heavy Metals Commonly Found in Water Bodies

These heavy metals are expected to be found in aquatic environments include. Lead, cadmium, mercury, and arsenic. These metals can be regarded as highly toxic and persistent in the environment and are able to bio magnify in aquatic organisms. Lead gets into waterways via industrial effluents and gasoline, to mention but a few, while cadmium enters waterways because of mining and phosphate fertilizers among others. The main cause of mercury pollutions is from industries sources and improper disposal of wastes while arsenic is more geochemical in origin augmented by anthropogenic factors including the use of pesticides [8].

1.3.3 Mechanisms of Heavy Metal Uptake in Fish

Heavy metals enter the fish body through gills, skin, mouth and digestive tract any route associated with the ingestion of food. Due to the high surface contact with the environment, gills are effective organs in the uptake of metals from water. Metals adsorb onto the gill tissues and then within blood circulation, they go to other vital organs [9]. Metals consumed through food or water containing the elements can be transported through the gastrointestinal tract. Further, dermal kind of absorption occurs through contact with contaminated water. It is established that the

bioaccumulation of heavy metals spurs various factors such as the types of fish, age, size, concentration, and the time the fish spends in contact with heavy metals [10].

1.3.4 Bioaccumulation and its effects

Bioaccumulation is the process where certain chemicals, such as heavy metals, build up in the tissues of living organisms at levels higher than those in the surrounding environment. Drinking tainted water, eating aquatic foods that have absorbed heavy metals, or coming into touch with contaminated water on the skin can all expose humans to these dangerous compounds. Metal bioaccumulation depends on metal solubility, environmental conditions and biological status [11].

1.3.5 Factors influencing Bioaccumulation

Heavy metal bioaccumulation in fish is influenced by various factors, including the nature of the metal, its chemical state and the surroundings. Moreover, there are biological features of fish species, age and diet that contribute to the effect. For instance, young fish tend to assimilate metals more effectively because they have higher metabolic rates and greater surface area-to-volume ratios. Additionally, higher trophic level fishes which are mainly predators tend to accumulate relatively high levels of metals due to bio magnification [12].

1.3.6 Toxicity of heavy metals

The toxicity of heavy metals differs and can modify fish physiology, behavior and survival ratio. Lead, cadmium, mercury as well as arsenic are sorts of metals that can affect the liver, kidney, gill, muscle and other vital organs in the body. These changes cause cellular or tissue dysfunction since these metals interfere with enzyme-catalyzed reactions; metabolic pathways end up inducing oxidative stress. Continuity of exposure leads to their death more frequently since heavy metal affects growth, and reproduction. The presence of heavy metals in aquatic environments can disrupt essential behaviors in fish, including their ability to feed efficiently, evade predators, and engage in successful mating activities [13].

Fish accumulate heavy metals in their bodies that later lead to significant physiological and pathological transformations in different vital organs. Due to their involvement in key physiological functions such as metabolism, excretion, respiration, and movement, these organs are particularly vulnerable to

bioaccumulation compared to others. Properly understanding how heavy metals interact with these crucial organs is essential in determining the overall health status of populations of fishes inhabiting polluted ecosystems [14].

Fish species liver serves as the primary site for detoxification and metabolism; hence easily accumulates large amounts of metallic elements ions or particles within its tissues. The basic activities carried out by cells become interrupted due to reactive oxygen intermediates generated from redox processes associated with oxidative damages caused by toxic elements like mercury cadmium as well as lead which may result into cell death [15]. Changes found in the liver such hepatocyte degeneration fibrosis inflammation are among common signs used for indicating whether a particular fish is suffering from poisoning because if it occurs it may lead to reduced detox capacity thereby lowering body condition.

Among the major targets for Heavy metal s toxicity are kidneys which play a role in osmoregulation plus excretion functions within fishes. Generally renal tissues get congested with accumulated metallic substances causing nephron & tubular damage. This in turn leads to impaired filtration, water balance and excretion ability of fish. In heavy metal-exposed fishes' common histological alterations seen include tubular necrosis, glomerular degeneration and interstitial fibrosis. The overall effect of chronic kidney damage is the retention of metabolic waste products which further increase toxicity within Fish system [16].

Gills are used by fishes for respirational purposes & they tend to be in direct contact with water hence making them more vulnerable to heavy metals as compared to other parts of the body. Gill tissues get damaged by metals resulting in functional and structural dysfunctions. Damage to the gills caused by heavy metals can result in conditions like lamellar fusion, hyperplasia, and epithelial lifting. As a result, the efficiency of gas exchange is compromised, leading to respiratory distress, hypoxia, and osmotic imbalances when the gills are no longer functioning properly [17].

These elements also can accumulate in muscles leading to poor quality or health condition of fish. The muscle atrophy, necrosis, and low protein content that results from mercury or cadmium accumulation is always observed in animals indicated above. Their swimming abilities growth, and survival rates all have been affected a lot due to different life forms' accumulation of these ions within their bodies.

Additionally, this raises concerns about the safety of human beings who consume fish with such metals stored in its tissues because most people eat flesh [17].

1.3.7 Confined vs. Natural Water Bodies

These water bodies include ponds and tanks, which are typically tight knit, however real or man-made. As a result of pollutants being concentrated within the confines of these environments and reduced dilution capacity, they often become heavily polluted. The presence of heavy metals with bioavailability depending on temperature fluctuations, pH changes, and dissolved oxygen levels is another issue that can occur in confined water bodies [18].

On the other hand, natural water bodies such as rivers and lakes have extensive surface areas and usually undergo natural flows and mixing. Because they undergo constant renewal as well as diluted effect which results in contaminated lower concentrations, unlike others. Nonetheless, pollution still occurs in natural water bodies resulting in high accumulation levels of heavy metals, particularly in places with high industrial or agricultural activities [19].

The difference between confined environments and open systems can be observed by comparing the bioaccumulation pattern of heavy metals in fishes from both sources. In confined habitats, fish tissues tend to accumulate more metal pollutants due to limited water exchange systems and higher concentration rates among contaminants than that experienced in free waters. Conversely, when it comes to freshwater ecosystems several mechanisms reduce metal concentration through dilution processes occurring from continuous flow of water thereby leading to lower levels of bioaccumulation effects than that found in marine ecosystems. However certain factors like point source contamination or local environmental conditions might affect bioaccumulation patterns within different types of aquatic systems [20].

1.4 Significance of the Study

There are several reasons why it is important to know about heavy metals effects on major carps. This is because they are very critical in terms of economic values in aquaculture and fisheries; hence, their health conditions have a direct effect on food security and livelihoods. Major carp are also important for maintaining the balance of aquatic ecosystems. Moreover, understanding how heavy metals accumulate and their toxicological effects in these fish can help to identify potential risks to fish

populations as well as provide insights that would facilitate the adoption of management approaches aimed at curbing pollution while conserving aquatic biodiversity. The implications for aquaculture and fisheries management from this study are significant. Policymakers and practitioners can therefore take action by implementing measures that will reduce pollution through proper knowledge of bioaccumulation trends as well as toxic effects of heavy metals on major carps hence creating sustainability in fish farming methods. Also, there is a need for this information to be incorporated into regulations guiding water quality control both in aquaculture systems and natural water bodies. For assessing potential health risks associated with the consumption of seafood including fish species containing heavy metals as part thereof, an understanding is needed about bioaccumulation levels specifically among fishes used for human consumption.

OBJECTIVES OF THE STUDY

- To measure the concentration of heavy metals in the liver and gills of major carp in both confined and natural water bodies.
- To assess the histopathological changes in the vital organs of major carps exposed to heavy metals.
- To compare the bioaccumulation patterns and toxic effects of heavy metals between confined and natural water bodies.

CHAPTER 2

REVIEW OF LITERATURE

The levels of heavy metals in fish species that were taken from the Shahdara Bridge, Baloki Headwork's, and Sidhnai Barrage, three important fishing locations along the River Ravi, over a single year, from June 2009 to May 2010 [21] examined. The concentration of heavy metals, such as aluminium (Al), arsenic (As), barium (Ba), chromium (Cr), nickel (Ni), and zinc (Zn), in different fish organs, including the gills, liver, kidneys, intestines, reproductive organs, skin, muscles, fins, scales, bones, and fats, was the focus of the researchers' attention. According to the study, all these metals had levels that differed significantly among the three sites and varied seasonally. Fish of all nature showed high concentrations of Aluminum and zinc; the highest levels were found in the liver and kidneys and the lowest in muscles and fats. Among all sites, Sidhnai Barrage showed comparatively lower metal toxicity, while the highest contamination levels were at Shahdara Bridge. The findings indicated that the accumulation of these metals in fish organs is directly related to the contamination levels in their environment. The present study thus raises serious concerns regarding the eco-toxicity of River Ravi, where the contamination levels exceed the permissible safety standards for aquatic life and human consumption.

Edo, [22] found the concentrations of heavy metals deposited and absorbed in the water and planktonic biomass of the River Ravi in Pakistan, with a focus on Pb, Cd, and Cr in particular. At several river sampling locations, the concentrations of these metals have been examined in water samples together with plankton. The results depicted the water and planktons in the River Ravi as highly contaminated with heavy metals that pose a major threat to the aquatic ecosystem of the river. Bioaccumulation of these metals in plankton, the primary food for a host of aquatic animals, suggests building up or magnification up the food chain to ultimately harm the fish and human health by consuming such fish. The study thus shows that the environmental management and pollution control strategy is very necessary for the reduction of heavy metals being discharged into the River Ravi to control aquatic environmental pollution.

Shafi et al. [23] evaluated the water quality regarding heavy metal contamination in River Ravi. In the research investigation, water samples were gathered and examined for many physio-chemical characteristics, such as the levels of heavy metals such as lead (Pb), cadmium (Cd), and chromium (Cr). Results showed that the water of River Ravi is highly polluted, and heavy metal levels were higher than prescribed standards for aquatic life as well as human consumption. The study provides proper insight into the sources of contamination and their possible impact on the ecosystem of the river. It stressed that effective strategies relevant to pollution management are urgently required for ongoing contamination and to prevent further degradation of the river's water quality. In addition, it is suggested that contamination by heavy metals poses serious risks to local communities who use the river as their water and fishing supply.

Mahmood et al. [24] analysed water samples from river Ravi to determine their physicochemical qualities as well as their heavy metal toxicity. The researchers conducted a comprehensive analysis of the water samples, measuring dissolved oxygen, pH, temperature, and concentrations of heavy metals including chromium, lead, and cadmium (among others). The quantities of heavy metals are discovered to be greater than the permitted criteria for safe water, indicating a noticeable deterioration in the water quality of the Ravi River. Considering the findings of the research, immediate action is required to remedy pollution and safeguard the ecology of the river as well as human health

Jabeen and Chaudhry [25] examined how fish species from the River Indus in Pakistan were bio accumulating heavy metals (cadmium, lead, chromium, and zinc). In this regard, extensive water, sediment, and fish tissue (liver, gills, and muscles) sampling carried out during different seasons. The metal concentrations for ascertaining health hazard in human consumption of such fishes through Atomic Absorption Spectrophotometry were determined. The two most selected metals were zinc and lead, and they were the most accumulated. The prevalence was high in the liver and muscles. The great accumulation differences of the metals were contributed by seasonality, where the concentration was higher during the dry season than the wet season. Jabeen and Chaudhry [25] suggested that the metal contamination in River Indus should be periodically monitored with a view to strategies for pollution control in order to minimize the risks.

Malik and Zeb [26] assessed the extent of heavy metal contamination in Pakistan's Soan River, with particular attention to the bioaccumulation of lead, zinc, cadmium, chromium, and copper by different fish species. The methods involved analysis of composite water, sediment, and fish tissue samples collected from various points on the bank of the river. Atomic absorption spectrophotometry was used to detect the metal content in the fish's muscles, gills, and liver. Results showed that high concentrations of cadmium and lead were above the tolerance limit values for use as human food, particularly in the liver and gills. The good values of correlation obtained for metal concentrations in sediments and their bioaccumulation in fish tissues have shown that sediment contamination in the river played a big role in the ecosystem. It is revealed from health risk assessment that the study shows very critical health hazards of consuming fish from River Soan, where immediate remediation efforts should be employed to reduce metal pollution.

Arain et al. [27] assessed the concentrations of the following heavy metals in the water, sediments, and fish species tissues from Keenjhar Lake, Pakistan: Lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), and zinc (Zn). The samples were drawn from different sites of this lake, after which the samples were subjected to atomic absorption spectrophotometry. They showed, in two fish species, that metal bioaccumulation in tissues was differential with respect to muscles, liver, and gills based on their concentrations. It was similarly observed that the highest accumulation of zinc and nickel was at maximum levels, especially so in the liver, always above recommended safe limits prescribed by the World Health Organization. The research points out possible health risks for local populations, who rely on these fish as their chief source of protein, and presses for stringent measures in controlling industrial discharges, with regular monitoring for metal contamination in the lake.

Begum and Hari Krishna [28] examined how heavy metals including zinc, copper, lead, chromium, and cadmium bio accumulate in the organs of freshwater fish *Labeo rohita* collected from the Tungabhadra River of Karnataka State in India during 2009. The results shown assayed metal concentrations for liver, gills, and muscles of fishes by atomic absorption spectrophotometry. It concluded that among the main accumulated metals were zinc and lead, mostly in the liver, besides cadmium and chromium. The researchers have flagged off their study as proof of a possible health

risk from fish in the river, laden with cadmium and lead. The authors demanded immediate action on measures to control industrial effluents and attempts to reduce heavy metal contamination in the river.

Gupta et al. [29] Investigated the presence of heavy metals (Cu, Cr, Cd, Pb, Zn) in riverine water, sediment, and the muscles of two species of catfish that were obtained from the Ganges River at Allahabad. The two species of catfish were *Channa punctatus* (*C. punctatus*) and *Aorichthys aor (A. aor)*. According to the findings of the research conducted on water, the relative abundance of heavy metals was discovered to be as follows: Zn > Pb > Cu > Cr > Cd. According to the investigation of heavy metals in sediment, Zn, Pb, Cr, Cu, and Cd were the five heavy metals that accumulated to their maximum in sediment first, followed by the five heavy metals that were analysed. Heavy metals were found to accumulate in fish muscles in a sequence that was found to be comparable to that which was found in soil and water. This pattern was Zn > Pb > Cu > Cr > Cd. Zinc, in comparison to other elements, appears to have accumulated at a higher rate in both the sediment and the muscles of both fish species, as shown by the results.

Zhang et al. [30] investigated heavy metal contamination in 19 distinct fish species from the Three Gorges, Yangtze River, and Banan regions of Chongqing. Except for zinc in the top layers, the results showed that the mean levels of heavy metals were higher in the stomach than in muscle. In the muscle and stomach of fish residing in the top layers, there were considerable fluctuations in the mean concentrations of As, Cr, Cu, and Hg ($P \leq 0.05$). The mean Cr and Cu concentrations in the muscles and intestines of the fish residing in the intermediate layers were noticeably different. Fish residing in the lowest layers, however, showed considerably differing mean As, Cd, Hg, Pb, and Zn concentrations in their intestine and muscular tissues ($P \leq 0.05$). Compared to the upper strata (US) and middle strata (MS), the concentrations of As, Cd, Cr, Cu, Hg, and Pb in the muscle and gut of fish from the bottom layers (BS) were higher, although a higher concentration of Zn was found there. It was discovered that the mean metal concentrations in *Coreius heterodon* were higher at age II than at age I (2- and 1-year old fish respectively). Overall research revealed that fish muscle in the Banan region had just a little amount of heavy metals exceeding Chinese food standards.

Ali & Khan, [31] assessed The levels of heavy metals Cr, Ni, Cd, and Pb in the water, sediments, and several freshwater fish species of the Kabul River, Pakistan. The heavy metals in the samples were measured using an Atomic Absorption Spectrophotometer. Heavy metal contamination in fish muscle was evaluated using the metal pollution index and biota-sediment accumulation factor, while the danger to human health was determined by calculating the expected weekly consumption. The mean amounts of Cr, Ni, Cd, and Pb in muscle samples of the examined fish species at various sampling locations throughout the river varied from 12.3 to 33.0 mg kg^{-1} (Cr), 33.2 to 109.2 mg kg^{-1} (Ni), 0.98 to 1.5 mg kg^{-1} (Cd), and 13.9 to 29.6 mg kg^{-1} (Pb), respectively. The current study indicates that the intake of the examined freshwater fish species from River Kabul is usually safe for possible risks from cadmium (Cd) and lead (Pb); nonetheless, the detected buildup of nickel (Ni) may provide a potential health risk to frequent or excessive fish eaters.

Nawaz et al., [32] conducted study to compare The concentrations of several dangerous substances in fish from that site to an adjacent aquaculture facility. Concentrations of As, Cd, Cu, Pb, Hg, and Zn, together with the electrolytes Ca, K, and Na, were quantified in several edible and non-edible freshwater fish species. Fish specimens were collected from two selected sites and analysed for their constituents. Contrary to typical concentrations of Zn (37.85-40.74 ppm) and Cu (1.39-2.93 ppm), elevated levels 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 detected. Mercury concentrations were significantly elevated in all assessed groups, and increased levels may induce coughing, lung dysfunction, and psychotic manifestations. Significant levels of toxic metals have been detected at study sites, necessitating more surveillance.

CHAPTER 3

MATERIALS AND METHODS

3.1. Field Sampling

Sampling of *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala* was done from December 2023 to February 2024 through a systematic sampling at three sites in Punjab, Pakistan. The choice of sites was focused on their regional water management standing and preclusion for fish habitat. The first site is Sulemanki Headwork's, situated on the River Sutlej near Sulemanki Village and approximately close to Depalpur City in the Okara District. This site is famous for its strategic value in water regulation and irrigation. The second site is related to the Balloki Headwork's site, also based on the Ravi River but located in the Kasur District. It was one more important facility for waterworks supporting irrigation and water management at the regional level. The third site encompasses the Fishponds of UVAS maintained at Ravi Campus Pattoki in the District Kasur. It's for this reason that the controlled environment ponds come in handy and relevant for serving as a reference point for the other two sites.

Soon after collection, the samples of fish were weighed for their physical features. The ice boxes helped to stop deterioration of the samples to maintain their freshness during transportation for the sake of carrying out various studies. Samples were brought to the Anatomy and Histology Lab of Histology at UVAS Main Campus Lahore for in-depth histology examinations.

3.2. Histopathological Analysis

Samples of liver and intestine were obtained each month from *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala* were collected at each of the three sites: UVAS-Fish Ponds, Sulemanki Headwork's, and Balloki Headwork's. The organs were excised and sectioned, then immediately fixed in fixative solution containing glacial acetic acid, formaldehyde, and ethanol in the ratio 1:3:7 according to the method described by [31]. This fixative solution ensured perfect preservation of the tissue architecture, a prerequisite for any histological study. The tissues were then dehydrated in graded ethanol and embedded in paraffin wax for precise sectioning. The paraffin-embedded

tissues were sliced into 2 µm thick sections using a microtome. These thin sections were stained with haematoxylin and eosin, which is one of the standard histological staining methods generally applied to enhance the cellular and tissue structures for better viewing under the light microscope. Sections were observed under a light microscope, and high-resolution photomicrographs were taken to document the histological features of the liver and intestine. This would have to include an analysis in search of any kind of change or anomaly of a pathological nature within the fish tissue and hence establish the health and physiological condition of fish under different environmental conditions.

3.3. Heavy Metal Analysis

Tissue samples from the liver and intestine of *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala* were analyzed at Government College University, Lahore. The first step was the preparation of tissue samples by washing them with deionized water to remove any possible external contaminants. The tissues were further dried with blotting paper to remove any moisture content. The dried samples were digested chemically to a solution using a mixture of 5ml solution of perchloric acid and nitric acid. The digestion is done at high temperatures of 200°C to 250°C in order to ensure that organic materials in the tissues are properly digested and that the heavy metals are released accordingly. The resulting solution was then diluted to volume with distilled water, preparing the sample for analysis. The concentrations of the different heavy metals were analyzed, which include Mn, Ni, Cu, Cd, Pb, and Cr, using an atomic absorption spectrophotometer. These are types of instruments that take advantage of the basic absorption measurements of light at characteristic wavelengths from specific metals to transduce the signals for quantification of the metals' concentration in tissue samples. This analysis was carried out following the Standard Methods for the Examination of Water and Wastewater [32]. Significant details regarding the bioaccumulation of heavy metals in fish were discovered, providing insight into the probable ecological and ecosystemic effects of pollution in these water systems.

3.4. Water Quality Analysis

Water quality assessment was done through the collection of a water sample in each site, whereby a litter of water was carefully put in water sampling bottles. In ensuring the integrity of the samples, the collection process was undertaken cautiously to ensure that no air bubbles are initiated that could compromise subsequent measurements. About two or three feet from the shoreline, the samples were collected just below the water's surface. On-site measurements for some of the key parameters with respect to indicators of water quality, such as temperature, conductivity, pH, total dissolved solids, and dissolved oxygen, were conducted using YSI digital meters. This provided full real-time representation of what the conditions were like at the time the samples were collected. The samples were taken to the Government College University Chemistry Lab in Lahore for further analysis targeting mainly metal content. Laboratory practices filtered all particulate matter out from the water samples, while 5ml of 55% nitric acid was added to every litter of filtered water so that metal ions would not stick on the surfaces of containers used for storage. After that, they were kept at 4°C until they will be ready for metal analysis; preserved water samples. It helped in ensuring reliability related to the data of water quality and a proper check on possible heavy metals, like Cu, Ni, Mn, Cd, Pb, and Cr. This is an important study, as the impact of alterations in water quality and environment at disparate sites on the health status of *Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala* was assessed.

3.5. Statistical Analysis

The data collected on water sampling were analyzed using a one-way analysis of variance (ANOVA) with PROC GLM in SAS software, examining variables such as fish, liver. Furthermore, a factorial ANOVA was conducted to assess the main effects of fish species and sampling sites, as well as their interaction. When significant differences were detected, Duncan's Multiple Range (DMR) Test was applied to compare treatment means, with statistical significance determined at $P \leq 0.05$. This approach allowed for a comprehensive evaluation of the relationships between the variables studied.

CHAPTER 4

RESULTS

4.1. Bioaccumulation analysis in Fish Liver

This study examines heavy metal accumulation in the liver of principal carps from diverse aquatic environments. The study seeks to compare the metal concentration levels in *Catla catla*, *Cirrhinus mrigala*, and *Labeo rohita* with a view to ascertaining species-specific differences. The concentration of six heavy metals (Chromium, Nickel, Lead, Manganese, Copper, and Cadmium) in the liver tissues of the three species is shown in Table 1. Of these metals, Nickel (Ni) showed a significant difference ($p = 0.006$) among species. Maximum Ni content was seen in *Cirrhinus mrigala* followed by *Catla catla*, whereas minimum was noted in Rohu, as indicated by different superscripts (a, b, c). Concentrations of Chromium (Cr), Lead (Pb), Manganese (Mn), and Copper (Cu) were almost similar in all the species without any distinguishable differences ($p > 0.05$). Cadmium (Cd) concentration was varied, from maximum in *Catla catla* to minimum in Rohu, but not significantly different ($p = 0.33$). Overall, Nickel demonstrated a clear variation of liver uptake among the three species.

Table 4.1. Heavy metal concentration in liver tissues of different freshwater fish species.

Fish Species	Cr	Ni	Pb	Mn	Cu	Cd
Catla catla	0.27 ± 0.05	0.16^{b} ± 0.01	0.24 ± 0.03	0.12 ± 0.02	0.24 ± 0.04	0.17 ± 0.11
Cirrhinus mrigala	0.28 ± 0.05	0.18^{a} ± 0.01	0.25 ± 0.03	0.13 ± 0.02	0.25 ± 0.04	0.06 ± 0.01
Rohu	0.29 ± 0.04	0.15^{c} ± 0.04	0.24 ± 0.03	0.11 ± 0.01	0.24 ± 0.04	0.04 ± 0.01
P-value	0.814	0.006	0.891	0.582	0.931	0.330

Superscripts on different means within column differ significantly at $P \leq 0.05$

This section of the study looks at the metals concentrations in fish the liver of fish collected from three places: Balloki Headworks, Sulemanki Headworks, and a Fish Farm (UVAS). The goal was to find out how the weather at each site affects the amount of metal that builds up. Table 2 shows the levels of six heavy metals Chromium (Cr), Nickel (Ni), Lead (Pb), Manganese (Mn), Copper (Cu), and Cadmium (Cd) in the liver tissue of the prevalent carps from the places listed above.

The results demonstrate that the concentrations of Cr, Ni, Pb, Mn, and Cu are significantly different amongst the three sites ($P \leq 0.05$). This suggests that where the samples were taken has a big influence on how much metal accumulates. Sulemanki Headworks had the highest levels of all the metals: Cr, Ni, Pb, Mn, and Cu accordingly. This means it was the most polluted of the three. Balloki Headworks had the highest average metal levels, whereas Fish Farm had the lowest. This is probably because the water conditions were cleaner and more regulated. Lead (Pb) and Copper (Cu) were the metals that were most common and had the highest concentrations in Sulemanki and Balloki. This shows that both metals are more likely to build up in the liver of fish. Cadmium (Cd) was the lowest at all the locations, especially at the Fish Farm and Balloki Headworks. The difference in Cd levels between the locations was not statistically significant ($p = 0.4478$), which means that they were all low and the same.

The liver tissues of fish from Sulemanki Headworks had the highest heavy metal concentrations, particularly for copper and lead, whereas the Fish Farm samples displayed the lowest levels of pollution. These results show how water pollution affects fish' health and how important it is to keep an eye on the levels of metals in natural streams.

Table 4.2 Heavy metal concentration in liver tissue of freshwater fish species across different sites.

Site	Cr	Ni	Pb	Mn	Cu	Cd
Balloki Headworks	$0.26^{b} \pm 0.04$	$0.15^{b} \pm 0.02$	$0.25^{a} \pm 0.02$	$0.13^{a} \pm 0.02$	$0.27^{a} \pm 0.02$	0.04 ± 0.01
Fish Farm (UVAS)	$0.16^{c} \pm 0.04$	$0.05^{c} \pm 0.01$	$0.16^{b} \pm 0.07$	$0.07^{b} \pm 0.03$	$0.15^{b} \pm 0.09$	0.02 ± 0.02
Sulemanki Headworks	$0.38^{a} \pm 0.03$	$0.19^{a} \pm 0.01$	$0.30^{a} \pm 0.02$	$0.15^{a} \pm 0.03$	$0.30^{a} \pm 0.03$	0.13 ± 0.22
P-value	0.0007	≤0.0001	0.0038	0.0079	0.0082	0.4478

Superscripts on different means within column differ significantly at P≤0.05

Table 3 illustrates interaction effects of fish species (*Labeo rohita*, *Catla catla*, and *Cirrhinus mrigala*) and sampling locations (Balloki Headworks, Sulemanki Headworks, and Fish Farm UVAS) on heavy metal content in the liver. The content of six heavy metals, i.e., Chromium (Cr), Nickel (Ni), Lead (Pb), Manganese (Mn), Copper (Cu), and Cadmium (Cd), is shown in the table.

Although differences in metal concentrations can be seen between sites and fish types, the interaction between sites and fish type is not significant for any of the metals ($p > 0.05$). This suggests that the combined effect of location and fish type does not lead to a significant difference in metal concentrations in the liver. However, when isolated, fish of Sulemanki Headworks suggested the maximum deposition of all metals, particularly in *Cirrhinus mrigala*, suggesting increased levels of pollution at this natural site. The Fish Farm, however, showed lower metal content in all species due to presumably controlled and cleaner water conditions. Of metals, Lead (Pb) and Copper (Cu) appeared most common in concentration, especially at natural sites, whereas Cadmium (Cd) was lowest in all pairs. Though there were minimal variations across species at each site, these variations were not statistically different in interaction terms, indicating that species and location alone affect heavy metal accumulation in fish liver.

Table 4.3. Heavy metal concentration in liver tissues of freshwater fish species interaction among selected sites.

Sites	Species	Cr	Ni	Pb	Mn	Cu	Cd
Balloki Headworks	*Rohu*	0.28 ± 0.01	0.18± 0.02	0.25 ± 0.04	0.12 ± 0.003	0.28± 0.02	0.04 ± 0.01
	Catla	0.29 ± 0.04	0.15± 0.00	0.25 ± 0.02	0.13 ± 0.02	0.27± 0.02	0.05 ± 0.01
	Cirrhinus marigala	0.30 ± 0.01	0.17± 0.01	0.27 ± 0.02	0.14 ± 0.02	0.29± 0.002	0.05 ± 0.01
Fish Farm (UVAS)	*Rohu*	0.21 ± 0.12	0.14± 0.02	0.16± 0.06	0.07 ± 0.03	0.15 ± 0.08	0.02 ± 0.02
	Catla	0.14 ± 0.04	0.06± 0.001	0.16 ± 0.07	0.07 ± 0.03	0.15 ± 0.09	0.09±0 .01
	Cirrhinus marigala	0.15 ± 0.04	0.10± 0.02	0.17 ± 0.07	0.08 ± 0.03	0.15 ± 0.09	0.03±0 .001
Sulemanki Headworks	*Rohu*	0.38 ± 0.004	0.20± 0.001	0.30 ± 0.03	0.13 ± 0.01	0.30± 0.002	0.05 ± 0.001
	Catla	0.38 ± 0.03	0.19± 0.01	0.30 ± 0.02	0.15 ± 0.03	0.30 ± 0.03	0.29 ± 0.22
	Cirrhinus marigala	0.39 ± 0.01	0.20 ± 0.00	0.32 ± 0.02	0.16 ± 0.03	0.32 ± 0.02	0.07 ± 0.02
P-value		0.883	0.317	0.999	0.9948	0.999	0.334

Superscripts on different means within column differ significantly at $P \leq 0.05$

4.2. Bioaccumulative Levels of Heavy Metals in Fish Gills

The concentration of heavy metals in the fish gills at various sampling stations was extremely different from table 4. The fish collected from Sulemanki Headworks had the maximum concentration of Chromium (0.35 mg/L), Nickel (0.18 mg/L), Lead (0.29 mg/L), Manganese (0.13 mg/L), and Copper (0.28 mg/L) that indicates greater environmental pollution from this site. Moderate levels of these metals were observed at Balloki Headworks, while the lowest in fish were found at Fish Farm, suggesting controlled and cleaner conditions of water. Statistical analysis confirmed significant differences in the concentrations of Cr, Ni, Pb, Mn, and Cu between sites ($P \leq 0.05$). Cadmium, on the other hand, exhibited low levels that did not show significant variation ($p = 0.13$), suggesting a relatively consistent occurrence at all the sites. These findings place the gills in the foreground as metal exposure-sensitive indicators in diverse aquatic environments.

Table 1.4. Heavy metal concentrations in gills tissues of different freshwater fish species.

Site	Cr (mg/L)	Ni (mg/L)	Pb (mg/L)	Mn (mg/L)	Cu (mg/L)	Cd (mg/L)
Balloki Headworks	$0.26^{b} \pm 0.01$	$0.15^{b} \pm 0.01$	$0.23^{a} \pm 0.01$	$0.11^{a} \pm 0.01$	$0.25^{a} \pm 0.01$	0.04 ± 0.01
Fish Farm (UVAS)	$0.12^{c} \pm 0.02$	$0.12^{c} \pm 0.02$	$0.14^{b} \pm 0.03$	$0.06^{b} \pm 0.01$	$0.14^{b} \pm 0.04$	0.02 ± 0.02
Sulemanki Headworks	$0.35^{a} \pm 0.01$	$0.18^{a} \pm 0.02$	$0.29^{a} \pm 0.01$	$0.13^{a} \pm 0.01$	$0.28^{a} \pm 0.01$	0.05 ± 0.01
P-value	≤0.001	≤0.001	0.008	0.013	0.017	0.133

Superscripts on different means within column differ significantly at P≤0.05

The concentration of heavy metals in the gills of three freshwater fish species, i.e., *Catla catla*, *Cirrhinus mrigala*, and Labeo rohita, was studied to reveal species-specific accumulation of metals. Overall, Chromium (Cr), Lead (Pb), Manganese (Mn), Copper (Cu), and Cadmium (Cd) concentrations were found to be extremely similar in all three species without much difference ($p > 0.05$). As one can observe from Table 5, Nickel (Ni) was the only element where a significant difference ($p = 0.0496$) was observed, with the highest value being recorded in *Cirrhinus mrigala*, then *Catla catla*, and the lowest value in Rohu. These findings reveal that while most metals accumulate evenly among species, *Cirrhinus mrigala* is slightly more sensitive towards Ni accumulation by gill tissues.

Table 4.5. Heavy metal concentration in gills tissue of freshwater fish species across different sites.

Fish Species	Cr (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Cd (mg/kg)
Catla catla	0.24 ± 0.05	0.15[b] ± 0.01	0.22 ± 0.03	0.10 ± 0.02	0.22 ± 0.04	0.04 ± 0.01
Cirrhinus mrigala	0.25 ± 0.04	0.17[a] ± 0.01	0.23 ± 0.03	0.11 ± 0.02	0.23 ± 0.04	0.05 ± 0.01
Rohu	0.25 ± 0.04	0.13[c] ± 0.03	0.21 ± 0.03	0.09 ± 0.01	0.21 ± 0.03	0.03 ± 0.01
P-value	0.965	0.049	0.848	0.559	0.901	0.478

Superscripts on different means within column differ significantly at P≤0.05

Fish species and sampling locations were subjected to examination to evaluate the variability of heavy metal accumulation in gill tissues. Although there were small differences in metal concentrations between *Catla catla*, *Cirrhinus mrigala*, and Rohu at Balloki Headworks, Sulemanki Headworks, and the Fish Farm, statistical analysis showed that there was no significant interaction between any of the six metals (Cr, Ni, Pb, Mn, Cu, and Cd), since all p-values were more than 0.05. This indicates that combined effects of species and location did not have a significant effect on metal accumulation in gills. As clear from Table 6, the highest metal concentrations were invariably found in fish from Sulemanki Headworks with presumably higher levels of pollution at this site. Minimum concentrations were from fish farm, indicating better quality water under controlled conditions. Although with minor differences between species, the three fish species had relatively similar patterns of metal accumulation at every site, and there was no noticeable species-specific trend at the sites.

Table 4.2. Heavy metal concentration in gills tissues of freshwater fish species interaction among selected sites.

Site	Fish Species	Cr (mg/kg)	Ni (mg/kg)	Pb (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Cd (mg/kg)
Balloki Headworks	*Catla catla*	0.26 ± 0.04	0.14 ± 0.01	0.23 ± 0.03	0.11 ± 0.02	0.25 ± 0.03	0.04 ± 0.01
	Cirrhinus mrigala	0.27 ± 0.02	0.15 ± 0.01	0.25 ± 0.03	0.12 ± 0.02	0.27 ± 0.02	0.04 ± 0.01
	Labeo rohita	0.25 ± 0.01	0.15 ± 0.01	0.22 ± 0.01	0.10 ± 0.01	0.25 ± 0.01	0.03 ± 0.01
Fish Farm (UVAS)	*Catla catla*	0.12 ± 0.04	0.01 ± 0.01	0.14 ± 0.07	0.06 ± 0.03	0.14 ± 0.09	0.01 ± 0.01

	Cirrhinus mrigala	0.12 ± 0.03	0.01 ± 0.01	0.15 ± 0.07	0.07 ± 0.03	0.14 ± 0.09	0.01 ± 0.01
	Labeo rohita	0.14 ± 0.07	0.01 ± 0.01	0.14 ± 0.06	0.06 ± 0.03	0.13 ± 0.07	0.02 ± 0.02
Sulemanki Headworks	*Catla catla*	0.35 ± 0.03	0.17 ± 0.01	0.28 ± 0.04	0.14 ± 0.04	0.28 ± 0.04	0.05 ± 0.01
	Cirrhinus mrigala	0.36 ± 0.01	0.19 ± 0.01	0.30 ± 0.02	0.15 ± 0.04	0.29 ± 0.03	0.06 ± 0.02
	Labeo roita	0.35 ± 0.01	0.18 ± 0.01	0.28 ± 0.01	0.11 ± 0.01	0.26 ± 0.01	0.04 ± 0.01
P-value		0.969	0.850	0.999	0.982	0.999	0.958

Superscripts on different means within column differ significantly at P≤0.05

4.3. Physio-chemical Analysis of Water

Water quality plays a very important role in governing heavy metal accumulation in fish. Some of the most prevailing physio-chemical parameters such as dissolved oxygen, temperature, total dissolved solids (TDS), electrical conductivity, and pH have significant roles to play in metal accumulation as well as the overall health of the fish. During this study, there were notable variations in water quality between the three sampling sites. The Fishpond (UVAS) recorded the optimum water quality with the highest dissolved oxygen (3.50 mg/L), pH (6.60), and conductivity (1.26 mS/cm), which are indicative of well-controlled and managed conditions. Balloki Headworks recorded low water quality with the lowest dissolved oxygen (2.43 mg/L) and lowest pH (5.52). Sulemanki Headworks recorded the highest temperature (26.15°C) and mean values of other parameters. As shown in Table 7, all five water quality parameters were significantly different across the sites ($p \leq 0.0001$), confirming that all the sites exposed distinct aquatic environments that would be most likely to account for the different heavy metal accumulation recorded in the fish.

Table 4.3. Physio-chemical properties of water samples from three sites showing significant differences ($P \leq 0.05$) across all parameters.

Site	Dissolved Oxygen (mg/L)	Temperature (°C)	Total Dissolved Solids (mg/L)	Electrical Conductivity (mS/cm)	pH
Balloki Headwork	$2.43^{c} \pm 0.03$	$23.53^{c} \pm 0.03$	$136.33^{c} \pm 0.67$	$0.33^{c} \pm 0.01$	5.52^{b} ±

					0.01
Fishpond (UVAS)	$3.50^{a} \pm 0.03$	$25.83^{b} \pm 0.03$	$955.00^{a} \pm 2.89$	$1.26^{a} \pm 0.01$	$6.60^{a} \pm 0.03$
Sulemanki Headwork	$2.60^{b} \pm 0.03$	$26.15^{a} \pm 0.03$	$258.33^{b} \pm 1.67$	$0.53^{b} \pm 0.01$	$5.37^{c} \pm 0.01$
P-value	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001

Superscripts on different means within column differ significantly at P≤0.05

The concentrations of the heavy metals in water differed widely between the three sampling stations, and these represented different contaminations and environmental conditions. The highest levels of most metals were obtained at Sulemanki Headworks, and these included levels of Chromium (0.32 mg/L), Nickel (0.16 mg/L), Lead (0.23 mg/L), Manganese (0.11 mg/L), and Copper (0.24 mg/L), which indicate higher contamination levels. Balloki Headworks indicated moderate values, and minimum values of Cr, Pb, Mn, and Cu were observed at Fish Farm (UVAS) with improved water quality under controlled conditions.

As evident from Table 8, statistical tests ensured that the variances of Cr, Ni, Pb, Mn, and Cu at the sites were highly significant ($P \leq 0.05$) except for Cadmium (Cd), which was not significantly different ($p = 0.0874$) and was otherwise comparatively low at all sites. These findings indicate that fish living in natural water bodies, particularly Sulemanki, have a greater chance of exposure to increased amounts of heavy metals, which might affect their health status as well as metal bioaccumulation patterns.

Table 4.4. Heavy metal concentration in water from three different sites.

Site	Cr (mg/L)	Ni (mg/L)	Pb (mg/L)	Mn (mg/L)	Cu (mg/L)	Cd (mg/L)
Balloki Headworks	$0.21^{b} \pm 0.01$	$0.12^{a} \pm 0.01$	$0.20^{a} \pm 0.02$	$0.09^{a} \pm 0.01$	$0.22^{a} \pm 0.01$	0.03 ± 0.01
Fish Farm (UVAS)	$0.04^{c} \pm 0.02$	$0.06^{b} \pm 0.01$	$0.05^{b} \pm 0.01$	$0.02^{b} \pm 0.00$	$0.05^{b} \pm 0.01$	0.05 ± 0.02
Sulemanki Headworks	$0.32^{a} \pm 0.01$	$0.16^{a} \pm 0.01$	$0.23^{a} \pm 0.02$	$0.11^{a} \pm 0.02$	$0.24^{a} \pm 0.02$	0.04 ± 0.01
P-value	≤0.0001	0.0002	0.0008	0.0001	≤0.0001	0.0874

Superscripts on different means within column differ significantly at P≤0.05

4.4. Histomorphological Analysis

Different gill histology shows that heavy metal exposure damages fish. Fig. 4.1.A exhibits well-organized UVAS Fishpond gills with intact main and secondary lamellae. This shows that chromium (Cr), lead (Pb), and copper (Cu) contents were below EPA standards and that the gill tissues were uninjured. Balloki Headwork's gills have considerable histological changes (Fig. 4.1.B). Structure damage from high Cr, Pb, and Ni concentrations is shown by secondary lamellar fusion and capillary aneurysms. Metals that cause oxidative stress or direct toxicity may impair gill tissues and functioning. The gills indicate Sulemanki Headwork's maximum damage (Fig. 4.1.C). The intensity of lamella vacuolation and opacity showed significant epithelial cell damage. This may be because height heavy metals like chromium, lead, and manganese are greatest here. Extreme gill structural disruption from chronic metal exposure may impair fish breathing efficiency and health. These demonstrate that increased heavy metal contamination in aquatic environments damages fish tissue, emphasizing the need for pollution control. When liver tissue is treated with UVAS Fishpond, the pancreatic tissues (P.T) and portal vein (P.V) are usually intact. The low heavy metal level in water suggests that liver tissues from this place are healthy, as histological investigations indicate no significant changes. Photomicrograph in Balloki Headwork's liver tissue, Fig. 4.1.E shows portal vein (ABC) blood cell aggregation and hepatic cell vacuolation. The liver may react to high amounts of heavy metals like Cr and Pb that have strained cells at varying degrees. Sulemanki Headwork's portal vein congestion from blood cell accumulation is more noticeable in Fig. 4.1.F. Manganese and copper were the most plentiful

heavy metals in Sulemanki Headwork. Inflammation and liver impairment can result from blood cell congestion. Heavy metal pollution damages fish liver tissues and requires better monitoring and remediation in contaminated streams.

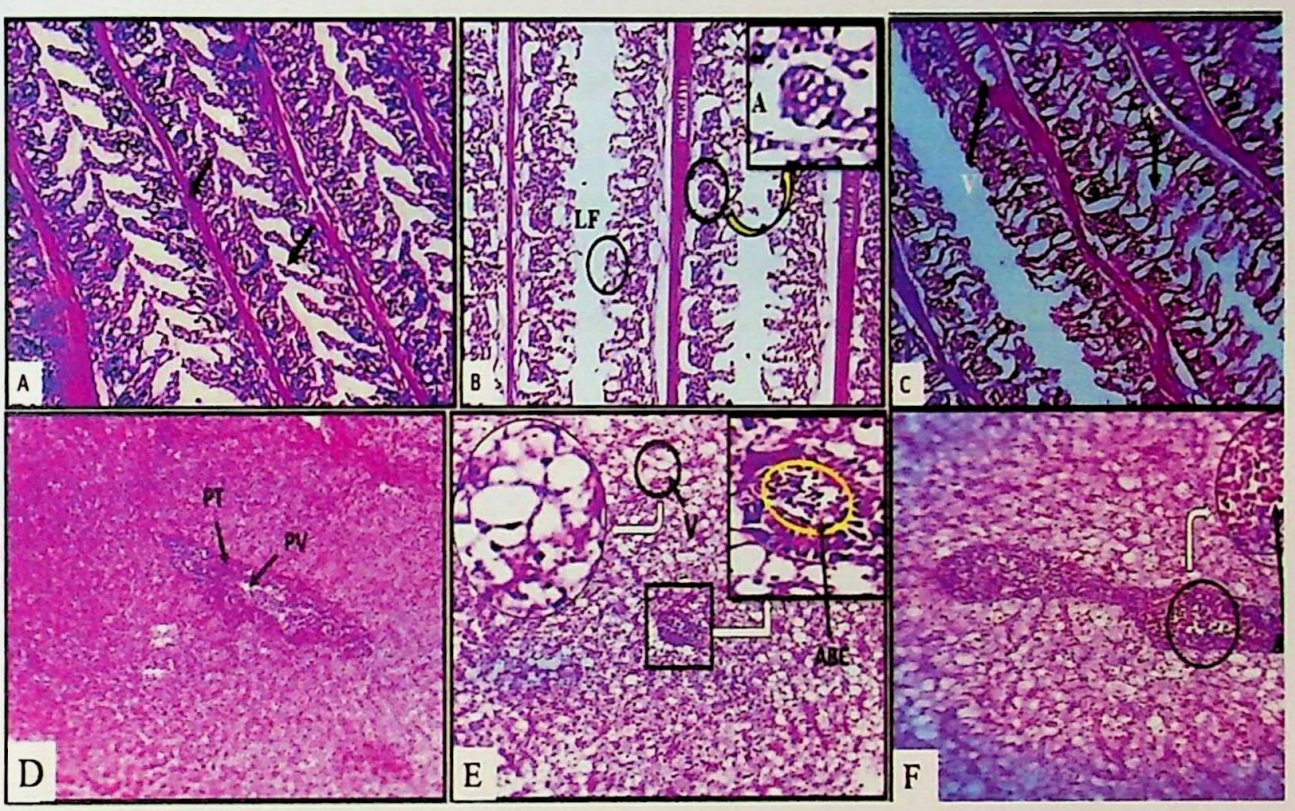


Figure 4.1. Histological sections of fish gills and livers show intact structures at UVAS Fishpond (A, D), while Balloki (B, E) and Sulemanki (C, F) exhibit lamellar fusion, vacuolation, aneurysms, and vascular congestion indicating progressive heavy metal-induced damage.

Gills histology demonstrates significant metal injury to fish. Fig. 2.A shows well-organized UVAS Fishpond gills with intact main and secondary lamellae. This reveals that gill tissues were unharmed, and chromium, lead, and copper amounts were below EPA guidelines. The gills of Balloki Headwork display significant histological alterations (Fig. 2.B). Secondary lamellar fusion and capillary aneurysms indicate Cr, Pb, and Ni-induced structure degradation. Oxidative stress or direct poisoning from metals can damage gill tissues. Gills show Sulemanki Headwork's greatest damage (Fig. 2.C). Extreme lamella vacuolation and opacity indicated epithelial cell injury. Perhaps this is due to the high concentration of heavy metals such as chromium, lead, and manganese in this area. Chronic metal exposure can severely damage fish gills, affecting respiration and health. These show that aquatic heavy metal contamination destroys fish tissue, stressing the necessity for pollution prevention. UVAS Fishpond generally preserves pancreatic tissues (P.T.)

and portal veins (P.V.) in liver tissue. Since histology tests show no alterations, the low heavy metal content in water signals healthy liver tissues. Photomicrograph Fig. 2.E shows clumps of blood cells in the portal vein (ABC) and changes in liver cells in the liver tissue from Balloki Headwork. High levels of chromium (Cr) and lead (Pb), which can strain cells, may negatively impact liver function. Sulemanki Headwork's portal vein congestion from blood cell buildup is more apparent in Fig. 1.F. In Sulemanki Headwork, manganese and copper predominated. Congestion of blood cells can cause inflammation and liver damage. Polluted streams need better monitoring and cleanup because heavy metal contamination affects fish liver tissues.

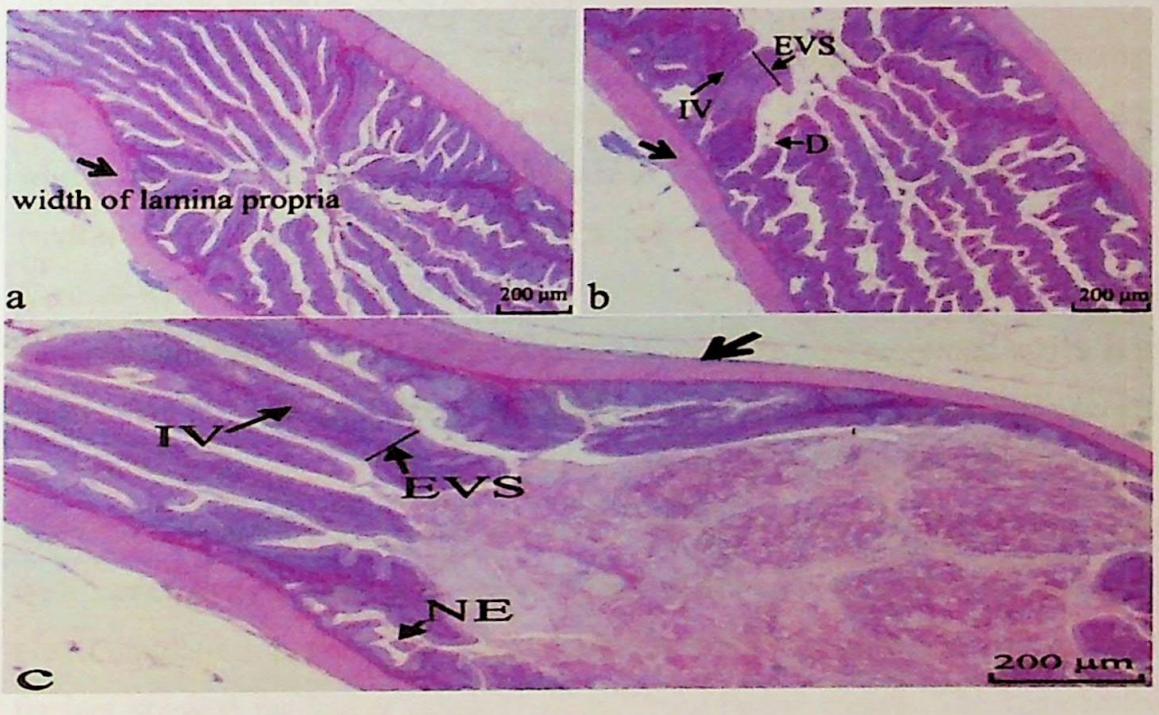


Figure 4.2. Histological sections of fish intestine show normal villi and lamina propria in UVAS Fishpond (a), while Balloki (b) and Sulemanki (c) exhibit epithelial vacuolization (EVS), disrupted villi (D), necrosis (NE), and increased inflammatory infiltration (IV), indicating heavy metal-induced mucosal damage.

CHAPTER 5

DISCUSSION

The present study aimed to evaluate the bioaccumulation of heavy metals and their impact on different organs of fish collected from 3 different freshwater bodies in the liver and gill tissues of three economically important freshwater fish species, *Catla catla*, *Cirrhinus mrigala*, and *Labeo rohita*. The liver contained increasingly higher concentrations of metals than the gills, a pattern consistent with the well-established liver role to detoxify and sequester metals [33]. Islam, [34] also found such findings, where in Indian major carps, hepatic tissue also contained higher concentrations of metals due to its active excretory and biotransformation role. Javed, [35] have also noted higher levels of hepatic metals in River Indus fish, further establishing the liver as a significant heavy metal accumulation site. This is also supported by [36], who pointed out that hepatic metal loads in fish are good indicators of long-term environmental contamination since they accumulate with time.

Species-wise comparison revealed that *Cirrhinus mrigala* had significantly higher concentrations of Nickel ($P \leq 0.05$) than *Catla catla* and *Labeo rohita,* which is most likely due to its bottom-foraging mode of feeding that exposes the species to sediment-bound metals. This is consistent with Malik, [37] observation, where they had found greater loads of metal in bottom-dwellers fish due to their constant contact with contaminated sediments. Shakir, [38] demonstrated similar species-specific bioaccumulation patterns in Bangladesh freshwater ecosystems, where benthic feeders showed far higher amounts of sediment-bound metals. The comparatively lower concentrations of metals in larger in-water feeding *Catla catla* and *Labeo rohita* are similar to the findings of [39] where metal exposure in column feeders and surface feeders was much more waterborne compared to sediment-related. However, non-significant difference in Cr and Cu among species shows that broader environmental exposure can mask the influence of species-specific biology in some cases, as previously suggested by [40].

Variability site-wise was staggering, with Sulemanki Headwork's being the most contaminated site with the highest concentration of metals in tissues. This spatial pattern is in accordance with that of [41], who indicated elevated heavy metal

concentrations in downstream regions of agricultural and industrial zones in Punjab's canal network. The abundance of metals like Cr, Pb, and Ni at Sulemanki is in harmony with [42] assertion that anthropogenic sources, especially from textile dyeing units, metal workshops, and agro-runoffs, contribute significantly to localized pollution. Compared to Balloki Headwork's receiving urban effluent, which also had intermediate pollution similar to the findings of [43]. UVAS Fish Farm, with controlled water quality, supported the lowest level of pollution, as has been emphasized by [44], who also mentioned that aquaculture ponds supplied with tube wells or purified water contain lesser loads of heavy metals. The steep spatial gradient emphasizes land-use activity and hydrological connectivity as the most important controls on contaminant profile development in freshwater ecosystems.

Water quality parameters were highly correlated with metal bioavailability and uptake in fish tissues. Higher water temperature and reduced pH in Sulemanki are essential as acidic pH increases the solubility of metal ions, making them more bioavailable, an aspect that is supported by [44]. Such physicochemical properties facilitate easier gill uptake and enhanced penetration across biological membranes. Higher EC and TDS values also indicate a higher amount of dissolved ions, potentially toxic metals [45]. The findings are consistent with [46] who established that high EC and TDS were directly correlated with water and fish tissue heavy metal concentrations. The more favorable parameters at the UVAS Fish Farm, with higher DO and close to neutral pH, also likely restricted dissolution and metal accumulation, as supported by [47] and it was deduced that near-neutral pH slows down metal mobility and bioaccumulation in aquaculture systems.

The relationship between fish feeding habits and exposure to metals was also indicated by the unambiguous accumulation profiles regarding species. *Cirrhinus mrigala*, the benthic feeder, possessed a much greater quantity of Chromium and Nickel, supporting the postulation that benthonic species are more prone to metal accumulation through direct contact and ingestion of contaminated sediments [48]. These same patterns were observed by [49] who found that River Chenab's benthos contained higher toxicant loadings of sediment-bound pollutants than pelagic fish. Although *Labeo rohita* and *Catla catla* had lower metal content, neither was entirely free of contaminants, hence evidence that exposure to metals through water continues to be a significant pathway. The findings support Rahman, [50] whose

study attested to the reality that both sediment and water pathways are avenues of exposure to metals, with species behavior and environmental factors dictating the predominant pathway. While feeding behavior has some explanatory power, statistically insignificant differences in the majority of the metals suggest biological differences are overridden under certain conditions by environmental pollution.

Cadmium was separated from the others by its pattern it had the lowest overall concentration and remained consistent across species and sites. This may result from its relatively limited use in local industry and processes and from its strong sediment affinity under conditions of near-neutral pH. These findings are in agreement with the studies of [51] which reported low Cadmium levels in controlled system fish and natural ones with pH neutrality. Similarly, low Cadmium mobility under such pH conditions has been demonstrated by [52] lending support to the conclusion that its water availability is restricted other than when pH drops considerably. Even in low concentration, Cadmium is a harmful metal of concern. As shown by Qadir, [53] sub-lethal exposure to Cd is also able to disrupt endocrine and reproductive processes in fish. Therefore, the long-term threat of Cd bioaccumulation, even though presently small, cannot be disregarded in current monitoring efforts.

Histopathological observations of this study are definitive biological evidence of association of cell damage in fish tissue with metal contamination. Sulemanki liver and gill tissue showed clear-cut lesions lamellar fusion, lifting of epithelium, and congestion of blood demonstrating metal-induced inflammation and stress. The findings concur with the report of [54] who observed similar hepatic and branchial injury in tilapia from polluted environments. Ahmad, [55] observed that vacuolization and necrosis of the liver tissues of fish treated with industrial effluents in River Kabul. That there was no significant histopathological change observed in fish from the UVAS Fish Farm also reiterates the relationship between exposure to metals and tissue damage. These structural changes, especially in *Cirrhinus mrigala*, help to highlight its potential as a sentinel species for ecological health monitoring, consistent with the findings of [56] which proposed benthic fish as the most appropriate bio indicators for aquatic pollution on a long-term basis.

Lastly, the lack of significant interaction effects between species and site indicates that metal accumulation is controlled independently by either fish physiology or environmental conditions but not by the two combined. Such an indication is in

agreement with the discovery by [57] on metal uptake across species in the Yangtze River, where they determined that location-specific controls dominated over species-specific responses in shaping bioaccumulation patterns. This suggests that while some species are more likely to accumulate based on their biology, environmental conditions most significantly, pollution levels are the primary drivers. Overall, the implication is that mitigation efforts need to be focused on restoring the environment and managing pollution, rather than species-specific treatments.

The research indicates notable geographical and environmental variations in heavy metal contamination across specific water bodies. This underscores unregulated agricultural and industrial practices present considerable risks to human health and lead to the pollution of water bodies. Environmental control is therefore crucial. Future research should concentrate on prolonged monitoring, species-specific toxicokinetic, and various remediation strategies, including phytoremediation and biofiltration, as well as rigorous policy enforcement. The protection of aquatic biodiversity and food safety necessitates careful consideration through collaboration among scientists, legislators, and environmental agencies.

CONCLUSION

This study concluded that heavy metal accumulation in freshwater fish is significantly influenced by environmental consditions with water quality playing a more critical role than species specific factors. Among the sites Sulemanki headwork's showed the highest concentration of heavy metals in both water and fish tissues, liver, gills and intestine. The UVAS fish farm under controlled condition exhibited the lowest metal level and minimal tissue alteration. Nickel exhibited main species-specific accumulation, especially in *Cirrhinus mrigala*, but lead and copper were the most consistently increased metals throughout all tissues. These results show how important fish are as accurate bio indicators of pollution in water. In order to preserve aquatic ecosystems and ensure the safety of food, they also emphasize the significance of enhancing environmental monitoring and pollution management techniques. Heavy metal pollution in aquatic environments is becoming a bigger problem. To address this issue, it is essential to conduct regular water quality assessments in specific locations, particularly in high-risk areas such as Sulemanki, where pollution levels are significantly elevated. Individuals will ascertain the subsequent steps once these tests facilitate the rapid identification of poisons. Stricter regulations for industrial and agricultural waste are also essential. Because throwing trash into rivers is one of the main causes of water pollution and environmental damage. As long as fish farms are well-run and closely watched, chemicals that are bad for fish will not get to them. This is a better way to eat fish that is also better for the environment and saves fish and other sea life. It helps with sustainable food practices and makes people healthy.

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