ASSESSING TOXICOLOGICAL IMPACTS OF WATER QUALITY ON FISH IN THE DASU REGION OF INDUS RIVER

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DEDICATION

In profound gratitude, I dedicate this thesis to my beloved family, whose unwavering support has been my greatest strength. To my esteemed teachers, your guidance has shaped my academic journey. A special tribute to my elder brother, Mr. Fazal Haq, for his unwavering encouragement and belief in my potential.

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ABSTRACT

The physicochemical analysis of water samples from eight sampling locations, including upstream and downstream areas of the Dasu Hydropower Project (DHPP) and selected tributaries, revealed compliance with World Health Organization standards for parameters such as pH, electrical conductivity, total dissolved solids, despite elevated turbidity levels. Similarly, concentrations of heavy metals in water samples, including aluminum, arsenic, barium, cadmium, cobalt, chromium, copper, manganese, molybdenum, nickel, lead, Selenium, Strontium and zinc, met WHO standards. Examination of fish samples from the same locations confirmed compliance of selected heavy metal concentrations with WHO standards, supporting the safety of fish consumption in the studied region. Noteworthy findings include the prevalence of nickel in fish kidneys, emphasizing its bioavailability, and a distinct order of metal accumulation in fish liver, with molybdenum exhibiting the highest accumulation. Further analyses revealed vulnerability of fish organs to metal accumulation, with the liver being most susceptible according to the Metal Pollution Index (MPI) (liver MPI: 8.14, kidney MPI: 6.71), particularly in tributaries. Additionally, oxidative stress analysis indicated adaptive responses in antioxidant enzyme activities, suggesting a link between higher metal pollution and bioaccumulation. These results underscore the need for continued research to understand ecological consequences and potential health risks associated with heavy metal bioaccumulation in aquatic ecosystems surrounding the Dasu Hydropower Project.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometry
Al	Aluminum
APHA	American Public Health Association
As	Arsenic
Ba	Barium
BAF	Bioaccumulation Factor
BDL	Below Detection Level
BOD	Biological Oxygen Demand
CAT	Catalase
CCCs	Criteria Constant Concentration
Cd	Cadmium
CDNB	1-Chloro, 2,4, Dinitrobenzene
CMCs	Criteria Maximum Concentration
Co	Cobalt
COD	Chemical Oxygen Demand
Cr	Chromium
Cu	Copper
CVD	Cardiovascular Disease
DHPP	Dasu Hydro-Power Project
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DS	Dry Season
DTNB	2,5, Di-Thiobis Tetra-Nitro-Benzoic Acid
EC	Electrical Conductivity
EDTA	Ethylenediamine Tetra acetic Acid
EPA	Environmental Protection Agency
GPS	Global Positioning System
GSH	Glutathione S-Transferase
GSSG	Oxidized Glutathione
GST	Reduced Glutathione

H2O2	Hydrogen Peroxide
HNO3	Nitric Acid
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
K2HPO4	Dipotassium Hydrogen Phosphate
KH2PO4	Potassium Dihydrogen Phosphate
Mn	Manganese
MN Test	Micronuclic Test
Mo	Molybdenum
MPI	Metal Pollution Index
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
Ni	Nickel
NW	Northward
NWQL	National Water Quality Laboratory
OECD	Organization for Economic Co-operation and Development
Pb	Lead
PCRWR	Pakistan Council of Research in Water Resources
pH	Power of Hydrogen
ROS	Reactive Oxygen Specie
SAR	Sodium Absorption Ratio
SEM	Standard Error of Mean
TDS	Total Dissolved Solid
USFDA	United State Food and Drug Administration
WAPDA	Water and Power Development Authority
WHO	World Health Organization
WQC	Water Quality Criteria
WQS	Water Quality Standard
WS	Wet Season
WWF	Worldwide Fund for Nature
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Overview

Water is a precious commodity on Earth and essential for all living organisms in its pure form, as it makes up the bulk of living tissues and is essential for sustaining life. It fulfills a crucial function in the global economy. Water covers nearly 71% of the Earth's surface, and all living things rely on it. It occurs on Earth in big water bodies like oceans and underground aquifers. Only a small amount, 1.6%, is underground, and even less, 0.001%, is in the air as vapor, clouds, and rain. 97% of the water on Earth's surface is in the oceans. Only a small amount, 2.4%, is locked in glaciers and polar ice caps. Another small portion, 0.6%, is found in other surface waters like ponds, rivers, and wetlands (Gorde & Jadhav, 2013). In addition to humans, all other living forms on Earth depend on freshwater resources. The use of water resources is made up of subsurface water (95–96%) and surface water (e.g., lakes, rivers, etc.) (3.5%) (Giordano, 2009). Water pollution is a notable global issue impacting both developed and developing nations. Rivers, in particular, are facing escalating risks of contamination, posing a significant threat to surface water supplies worldwide (Nabeela, et al., 2014). The quality of surface water is crucial for various purposes, including home, industrial, and agricultural usage, along with the sustaining diverse freshwater aquatic species. Natural and anthropogenic actions can diminish value of water resources, impacting their quality and making them less suitable for human consumption and toxic for aquatic life (Chaudhry & Malik, 2017).

Freshwater habitats cover just a fraction of the earth's surface in comparison to aquatic and land-based ecosystems. However, they hold great significance for humans as they provide an easy and cost-effective source of water for residential and farming needs. Water bodies such as rivers and lakes play a crucial role in providing clean drinking water, supporting agriculture, and serving various household requirements (Vidyasagar, 2007). Recent studies indicate that the supply of fresh water will be a significant challenge in the 21st century. Pollution of water poses a significant threat to the health of aquatic ecosystems. Many freshwater sources globally are experiencing pollution, leading to decline in water quality. Water contamination is a complex topic that involves a variety of substances stepping into the water cycle (Singh, et al., 2017). The level of pollution in surface water is easier to understand compared to groundwater. Due to human actions, the water gets filled with various nutrients that can lead to eutrophication and a decline in water quality. This may also result in an increase in harmful chemicals, a decrease in the area of water, and a decline in visual appeal (Bansal & Khare, 2000).

1.2 Water Quality of Indus Basin and Upper Kohistan District KPK

The Indus Basin faces significant difficulties in its water industry, stemming from factors such as increasing population, rapid urbanization, and manufacturing, damage to the environment, uncontrolled resource utilization, improper consumption of water, and poverty. These challenges are further exacerbated by the impact of changes in the climate. Four countries share the Indus Basin: Pakistan, India, Afghanistan, and China. Given the present population of 237 million people, which is expected to rise to 319 million in 2025 and 383 million in 2050, it is evident that water supplies are currently being fully utilized, with over 95% being used for agriculture (Malik, et al., 2010).

The riverine systems in Pakistan are experiencing increasing levels of pollution over time. One of the main factors contributing to this issue is the concentration of causes of pollution, including manufacturing, farming, and household operations, along the river corridor. These elements together contribute to the decline in the purity of water, raising concerns. As per the worldwide fund for nature (WWF), dams, water extraction, and climate change pose substantial threat to five rivers in Asia, impacting 870 million people. The Indus River is facing significant threats, putting one of the top five river systems at risk. Several investigations have been done to examine the quality of water in the Indus River (Rahman & Chughtai, 2014). Climate change is expected to lead to a temporary increase in the supply of water. Nevertheless, over time there will be a decline in water supply. Certain elements in the basin require a thorough reassessment. Over the last ten years, there has been an ongoing rise in water abstractions, particularly groundwater extractions. This is to sustain a rice-wheat structure, where rice and other crops are raised throughout the Kharif (moist, summer) period, and grain is raised during the Rabi (dry, winter) period. Nevertheless, the viability of this system in its present state is uncertain. There is a need for increased water supply for both residential and commercial purposes in the future. This can be achieved by reducing irrigation needs (Janjua, et al., 2021).

A study by Laghari, et al., (2012) has provided a detailed compilation and explanation of the various choices for effective water resource management inside the basin, both for the present and the future. Implementing sustainable water resource management actions involve incorporating strategies for managing the water supply and reducing the need for water. Alternatives for managing water supplies in this area involve various strategies. This involves managing reservoirs to account for the seasonal changes in water supply, conserving water quality and investing in wastewater facilities, exploring substitute water resources such as sewage recycling and desalting, and implementing development plans and soil-preserving measures. Additionally, flood mitigation efforts aim to reduce erosion and sedimentation while restoring ecological services like wetland regions and natural flooding areas (Laghari, et al., 2012).

Sabir, et al., (2017) conducted study on the water quality in the upper Indus Basin, located in northern Pakistan. They collected water samples from the Indus River and its tributaries to analyze various physical and chemical variables, including total alkalinity, hardness, anions and physical characteristics. In addition, The Perkin Elmer atomic absorption spectrometer was employed to assess selected components. The study found that the levels of different physicochemical parameters met the safety standards set by prominent international organizations such as the World Health Organization (WHO) and the national body Pakistan Environmental Protection Agency (Pak EPA) for both agricultural and potable water. The assessed samples of water demonstrated SAR value surpassing 1.3, by statistical methods, it has been determined that the amounts of specific parameters in the River Indus and its smaller rivers have experienced increase due to both natural and human factors. However, the quality of water in the River Indus and its connected rivers within the upper Indus basin is considered suitable and safe for consumption, as well as for local and irrigation purposes. As a result, this research promotes utilizing it for consumption, household, and agricultural needs in the upper Indus Basin (Sabir, et al., 2017).

In the southern region of Khyber Pakhtunkhwa, Pakistan, Khan et al. (2018) conducted an evaluation to investigate the impact of urban wastewater on the water quality of the Indus River. Water samples were collected from seven distinct locations along the Indus River during both dry and wet seasons. The assessment involved measuring various physical, bacteriological and chemical parameters to evaluate water quality. Significant variations in the water quality of the Indus River were observed both temporally and spatially. The amounts of pollutants in WS were significantly higher than in DS (Zafar, et al., 2017).

The water quality varied among the sampling areas, and the type of discharges being released into the river influenced it. The water samples surpassed the safe levels set by World Health Organization. Piper's examination showed that the water sampled from the seven collection points along the River Indus exhibited an alkaline nature. Based on the correlation coefficient assessments, certain physical and chemical parameters can be regarded as key factors in assessing water quality. Strong correlations ($r > 0.70$) were observed among these parameters and the majority of the other variables under investigation. The cluster analysis results indicate that the discharge point at Shami Road significantly contributes to contamination, adversely impacting the water quality of the River Indus. Establishing wastewater treatment plants at all discharge points along the River Indus is imperative to preserve the quality of this vital freshwater resource in Pakistan, as highlighted by Khan et al. (2018).

1.3 Contamination of River Waters

Transition metals are commonly found in aquatic environment and they can adversely impact both aquatic organisms and human health, if their amounts surpass certain limit levels. The issue of water contamination brought on by transition metals, primarily originating from industrial production and mining operations, is currently attracting significant worldwide concern (Subramanian, 2004). Insufficient sewage treatment facilities and extensive commercial manufacturing in South Asian countries, including Bangladesh, India, Pakistan, and Nepal, frequently result in the contamination of lakes and rivers by transition metals. The rivers in the vicinity of Dhaka, the capital of Bangladesh, are particularly affected, showing significant pollution with iron (Fe), manganese (Mn), nickel (Ni), zinc (Zn), copper (Cu), and lead (Pb), leading to considerable harm to the ecological system. Similar contamination is observed in the aquatic environments of India, Pakistan, and Nepal (Prasad et al., 2020).

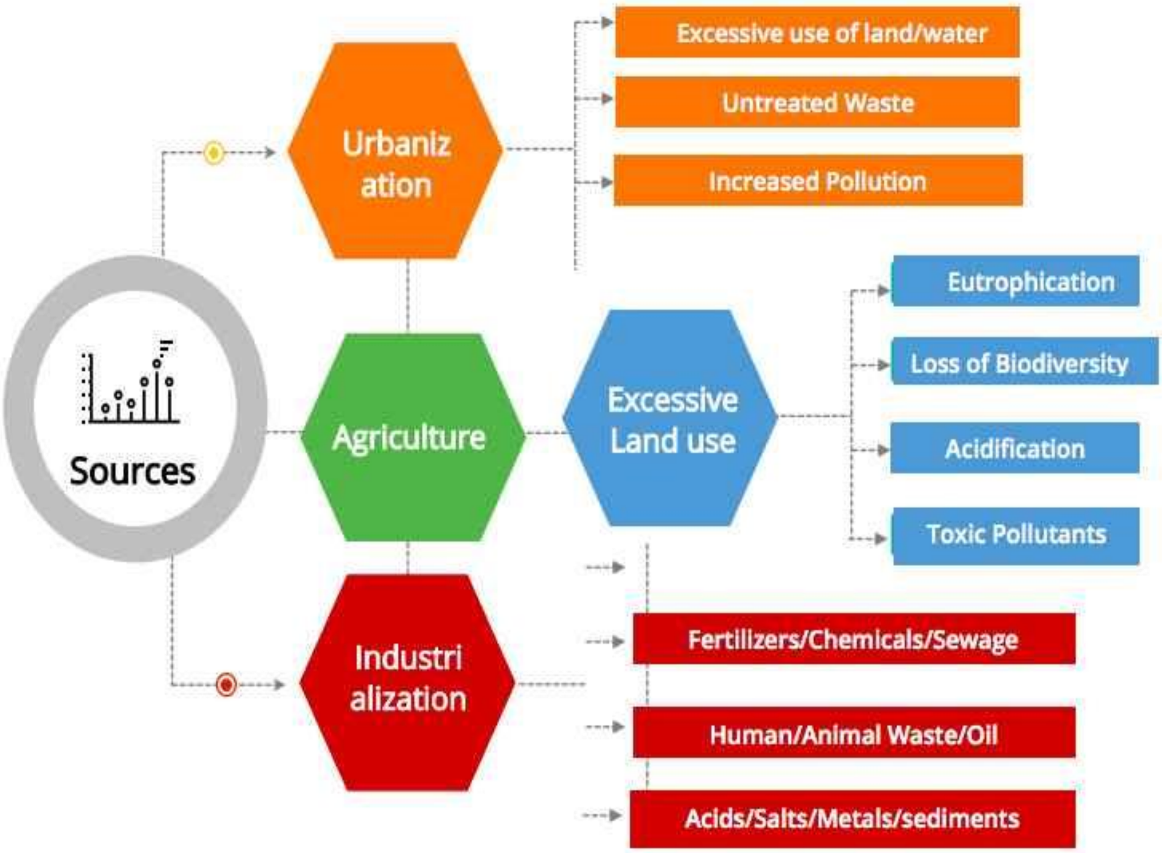

Figure 1.1: Major pollutants along with their source.

WQC (Water quality criteria) refer to the recommended optimal limits for chemical compounds or environmental factors, with the goal of safeguarding specific water resource activities and species from adverse consequences. Numerous countries, including the United States, Canada, Australia, and China, have established their distinct WQC systems, employing either dual or single threshold methodologies (Friberg et al., 2000). Within the realm of water quality criteria WQC, dual threshold values cover both chronic and acute criteria, employing criteria constant concentrations (CCCs) and criteria highest concentrations (CMCs) as pivotal limits to evaluate the immediate and prolonged impacts on aquatic species. However, countries such as

Bangladesh, India, Pakistan, and Nepal are yet to embrace these WQC standards, primarily due to challenges posed by Trans boundary Rivers (Wu et al., 2012). The primary river basins in South and Southeast Asia, namely the Brahmaputra, Indus and Ganges, originate from the region of Himalayas while provide essential resources to a population exceeding 1 billion peoples. These rivers serve as conduits between several nations, such as the River Indus basin encompassing three countries like Afghanistan, Pakistan and India. Bangladesh, Bhutan, India, and Nepal are interconnected through the Brahmaputra and Ganges basins. Shared river basins in South Asia have been the subject of controversies in the past few years due to concerns about the cross-border migration of hazardous contaminants. A Water Quality Certification (WQC) specifically for South Asia, rather than for individual countries within the region, is crucially required to safeguard and evaluate the ecological hazards of aquatic ecosystems (EPA, 2010).

Environmental contaminants such as a diverse range of natural and human-caused sources contribute to the release of toxic metals into the surrounding. Vehicle traffic emerges as a key human-induced source of heavy metals, including zinc (Zn), chromium (Cr), cadmium (Cd), and lead (Pb). Assessing the ecological health of river water is crucial for safeguarding aquatic biodiversity (Begum, et al., 2006). Analyzing the levels of heavy metals in fish body is crucial for public health concerns due to their ingestion by people. Biological processes do not require metals like lead (Pb) and Cadmium (Cd). Specifically, cadmium is recognized as the most poisonous element (Aslam & Yousafzai, 2017). A recent investigation conducted in China on a substantial population of adults has discovered a correlation between blood Pb levels and a higher occurrence of heart illnesses (CVD). In addition to effecting fish consumers, toxic metals also impact fish exposed to them. The existence of heavy metals in fish hinders the release of hormones, which promote reproduction and lead to pathological alterations. Chromium induces biochemical, hematological, and behavioral alterations in fish. Research has revealed that the fish's locomotion behavior was altered as a result of exposure to Cr (Ebrahimi & Taherianfard, 2011). Furthermore, it induces detrimental consequences such as alterations in physical structure, tissue death, and recurring bleeding in the gills of the fish that are subjected to it. Studies have indicated that tilapia fish exposed to low levels of Pb can potentially inhibit their immune system. The process of development and urbanization, along with rapid growth in population and economic growth, has led to the decline in environmental quality and the pollution of freshwater habitats with a range of harmful compounds, especially heavy metals. Rivers are aquatic ecosystems that exhibit high susceptibility to environmental fluctuations and contamination. The River Shah Alam is a highly contaminated stream of the River Kabul, receiving effluent from various businesses like distilling facilities, oil mills, paper factories, sugar plants, tanneries, and textile factories (Ebadi & Hisoriev, 2017).

Water contamination in Pakistan has emerged as a significant issue due to the rapid growth of industrialization. Rivers are constantly receiving discharges of industrial waste and residential wastewater. These releases include hazardous heavy metals. The flow of pollutants into rivers in Pakistan has damaged freshwater fisheries. Currently, aquatic bodies are receiving commercially contaminated waters devoid of undergoing a prior treatment process. The fall in the population of the vulnerable freshwater species Tor Putitora inhabiting the Kabul River is due to marine chemical contamination (Javed & Usmani, 2016). Heavy metals have the potential to be harmful, with mercury, cadmium, and lead being particularly hazardous. Several factors, such as particles, soil erosion and drainage, air deposits of dust and aerosol, and releases of sewage, can contribute to buildup of toxic metals in aquatic organisms. Upon introduction into aquatic habitat, heavy metals became embedded in particles and gradually escape into the surrounding water. Sediments play a crucial role in storing heavy metals in aquatic environments (Singh, et al., 2014).

Polluted water with contaminants and toxic metals is discharged from factories. The polluted state of this water is a major contributor to aquatic problems. The excessive use of pesticides in aquatic environment has led to issues for aquatic plants and animals. The water system plays a crucial role in Pakistan's economy. Observing the health of the fish within it can assess the health of a water environment. Aquatic creatures accumulate contaminants from both the water and their food sources. Placing contaminants in water negatively impacts the health of fish and other aquatic creatures. Consequently, the dwindling catch has led to significant financial losses for fishers. To maintain the health and safety of people, it is crucial to study and comprehend the effects of pollutants on the water bodies (Deng, et al., 2017).

Around the world, water bodies are mostly polluted by heavy metals, which cannot be broken down by living organisms. Heavy metals harm the environment by disrupting different biological processes in animals and plants. The harmful effects of different heavy metals can slow down the growth, bodily functions, and ability to have babies in fish. It can also cause them to die. Many studies found that fish exposed to metals had problems with their immune systems and were more likely to die. Heavy metals make the damage to DNA in the body worse by either directly causing harm or by making other harmful chemicals more toxic (Swarup et al., 2006). As the population grew, so did the construction of buildings and metropolises. The contamination of water from sources such as residential areas, agriculture, and industrial facilities has resulted in significant concern for public health. Pollutants that dissolve in water from industries and cities seep into the ground and are then quickly carried to rivers, lakes, and oceans. Some of the toxin breaks down or becomes a gas, while the remaining turns into solid salts and mixes with the soil in the ground (Walia et al., 2015).

The occurrence of toxic elements in aquatic environment can be extremely detrimental. Lead is a type of heavy metal that can harm animals and plants in the water. It gets into the water from natural events and human activities. People worldwide are eager to understand the effects of harmful metals in water on fish (Chai et al., 2017). This is important because pollution in the water and fish farming are becoming more common. According to several researchers, lead nitrate has adverse effects on fish. Limited research has been conducted on the impact of lead nitrate on fish and their aquatic habitat. Fish rely heavily on the quality of water for their health and survival. It has an impact on their rate of growth, their wellbeing, and their survival rate. It also has an impact on the income generated from fishing and the overall well being of the community (Patra et al., 2007).

Figure 1.2: Different water pollutants that affect aquatic life from varied sources.

Water systems are often utilized for the disposal and recycling of contaminated water and waste, with any excess water being discharged into the ocean. There is more pollution, and more people are using water for development, farming, building, making things in factories, and generating electricity in power plants. This helps reduce the amount of water that people use (Bukola et al., 2015). The pressure of the water influences the life in the water. Fish are typically essential for people residing near bodies of water. The environment can be altered in a harmful manner by factors such as chemicals, leading to pollution. Water habitats are fragile and in danger because they are mainly affected by pollution from homes, cities, and factories. Different farming methods cause pollution to enter the river (Kakute & Murachi, 2002). In water environments, the most common pollutants are heavy metals and pesticides. Heavy metals pose a major concern in water due to their accumulation in the bodies of animals and their slow elimination. Pesticides used on farms go into the air in different ways, like when they are sprayed, when they evaporate, or when the wind blows soil around. These chemicals in the water can change how aquatic animals grow and survive (Velcheva et al., 2010).

1.4 Impacts of Contaminated Waters on fish populations

Water pollution is a significant problem that is worsening every day. People rely on fish as a source of food, but they may also contain toxins that pose health risks. Fishing is an important way for people to make a living and helps a country grow economically. It is an important source of good protein, vitamins, important minerals, and healthy fats for people. Globally, fish provides nearly 3.0 billion people with around 20 percent of their animal protein, and approximately 15 percent of the protein intake for about 4.3 billion people is derived from fish (Jabeen & Chaudhry, 2011). Most people who live near the coast rely on fishing for their jobs and to make a living. The issue of fewer fish is tough for people who depend on fishing for their income and food. The fish in Pakistan's polluted water are in danger, as people are using prohibited nets to catch them. Small fish are also caught in these nets. The quantity of fish being caught by fishers in the rivers of Pakistan is declining. In the Indus River system, there are 200 distinct types of animals. 32 of these animals are only found in Pakistan. Several bodies of water in Pakistan are experiencing the issue of fish vanishing (Fanning et al., 2011). The decline in the fish population in Swat district is attributed to several factors. Residents and fishers due to their poor health and visible injuries avoid the fish in the river Swat. Consuming these deceased fish could potentially result in illness. The newspapers also reported on the ailments affecting the fish in the river Swat. The river becomes more acidic and oxygen levels decrease when it is exposed to heavy rain and pollutants. Insufficient oxygen levels in the water, combined with other factors, increase the likelihood of fish mortality. In addition, research has shown that many microbes are responsible for the increased rate of illnesses in fish (Islam et al., 2017).

The majority of toxins originate from urban areas, industrial facilities, deserted military locations, and agricultural lands. They are thrown into rivers and harm the animals and plants that live there. Heavy metals are a big problem that can build up in the body because they do not impair easily and are difficult to eliminate from the body. Industrial activities constitute the primary source of heavy metal contamination in water

(Paul et al., 2016). The most polluting heavy metals are zinc, chromium, copper, and lead. There are two main layers of sediment in the ocean: one on the surface and one deep down. They interact with the ocean. Sediment pollution poses a threat to aquatic insects due to their proficiency in tolerating metals and frequent proximity to them. This makes the metal pollution worse (Garibaldi et al., 2004).

These species' ability to tolerate pollution could result in the spread of pollutants to higher levels of the food chain. As a result of these animals' enhanced capacity to accumulate Zinc and Chromium, the concentration of toxic metals in water bodies are lower compared than sediments, but greater in fish organs. Consequently, ecological monitoring can be employed to identify pollution and its detrimental impacts (Quaas et al., 2016). The micronucleus (MN) test is a widely utilized method for identifying genotoxic effects associated with environmental pollutants. The significance of this test is heightened in aquatic environments since it serves as the primary means of assessing the genotoxic activity of chemical substances. Fish are a suitable model for discovering Genotoxic substances in aquatic systems because they are susceptible to tiny levels of metals in water, are plentiful, and can live in a range of habitats (Abdel-Satar et al., 2017).

The fish are frequently positioned at the top of the aquatic food chain, allowing them to accumulate significant amounts of heavy metals from their diet, the water, and the sediment. The undesirable components in fish can counteract the beneficial components. Heavy metals in fish can harm people's health (Gomma et al., 2010). Many studies focused on the heavy metals found in the part of the fish that people eat. However, other research shows that metals are found in various parts of the body such as the liver, kidneys, heart, reproductive organs, bones, digestive system, and brain. Based on the books and studies, different kinds of fish store metal in their bodies in different ways. Many factors can affect how much metal fish absorb, such as their gender, age, and size, when they reproduce, how they swim, what they eat, and where they live (Khan et al., 2000).

Fish are the best at sensing harmful toxins in water because they are very sensitive to even small amounts of metals, are found in large numbers, and live in different places in water. Water creatures like fish can absorb harmful substances directly and then change these substances into even more harmful ones (Kumar et al., 2018). For instance, bacteria can change mercury into very harmful chemical called methyl mercury, which then gets into fish. Young fish, baby fish, and small fish are very sensitive to pesticides and pollution from heavy metals in the water. This has the potential to cause them harm. Changes in the important body parts such as the heart, lungs, and liver. The fish's gills, kidneys, and liver can affect how well it can survive, stay balanced in the water, and reproduce. If they are not functioning correctly, it could result in a decrease in fish and alterations in the population (Khoshnood, 2017).

1.5 Literature Review

The severity of transition metal contamination in rivers in South Asia surpasses that of other parts of the world due to insufficient implementation of freshwater management practices. There is a pressing need for water quality standards (WQS) throughout South Asia to safeguard regional aquatic habitats, particularly due to the presence of trans boundary waterways. The current research by Wang et al., (2022) employed nonparametric kernel density estimation to construct species sensitivity distribution models. Following this, the research aimed to establish the permissible hazardous concentration to protect 95% of all aquatic species and define water quality criteria (WQC) for six prevalent transition metals in South Asia. The results revealed a ranking of acute and chronic water quality criteria (WQC) as Mn > Fe > Cd > Zn > Cu > Hg and Cu > Fe > Cd, respectively. Additionally, a thorough assessment of the potential risks posed by these metals in major rivers such as the Ganges, Indus, Brahmaputra, Bagmati and Meghna was undertaken. The findings highlight substantial pollution levels from transition metals in the major rivers of South Asia, posing significant biological threats to numerous aquatic species. This study holds the promise of deepening our understanding of ecological hazards in South Asia and laying a scientific groundwork for revising water quality regulations and enhancing overall water quality standards (Wang et al., 2022).

Siraj et al. (2016) conducted a study to evaluate the deposition of heavy metals in various tissues of Wallago Attu fish from the River Kabul in Khyber Pakhtunkhwa, Pakistan. The objective was to quantify the concentrations of Ni, Zn, Cr, Cu, Mn, Cd, Fe, and Hg in different parts of Wallago Attu. Samples were collected from contaminated sections of the Kabul River for comparison with control fish captured from Warsak Dam. The findings indicated that the order of metal accumulation in the skin, gills, and muscles was Zn, Cr, Pb, Cu, Ni, Fe, Mn >, Hg, Cd. In the colon, the order was Zn, Pb, Cr, Cu, Ni, Fe, Mn, Hg, and Cd. In the liver, the order was Zn, Pb, Cr, Cu, Ni, Fe, Mn, Hg, and Cd. The distribution of metals in various tissues of W. Attu followed the order of skin > gills > gut > muscle > liver. The skin, being the primary organ of interest, highlighted that the method of metal absorption was a direct outcome of fish-to-metal contact. The liver exhibited the lowest metal concentration compared to other parts in the same fish (Siraj et al., 2016).

Ali et al. (2020) conducted a research project to examine the concentrations of Ni, Cr, Pb, and Cd in the sediments, water and various species of fish inhabiting River Shah Alam, a branch of Kabul River in Pakistan. The researchers collected diverse samples from the river, specifically below the city of Peshawar. These samples underwent analysis using atomic absorption spectrophotometry to detect the presence of specific heavy metals. An investigation was carried out to explore the accumulation of heavy metals in the muscle tissues of 6 distinct fish species sourced from the river. Moreover, the accumulation of heavy metals was examined in different organs, such as gills, skin, liver, and kidneys of two fish species. The muscle samples of the six distinct species of fish exhibited unpredictable fluctuations in metal content. The interspecies variations did not show statistical significance, likely because there was a higher level of variation within the samples. The metal deposition in the five distinct organs of fish species does not exhibit the same pattern throughout the examined organs (Ali et al., 2020).

The occurrence of toxic metals in aquatic bodies is a sincere concern for the environment. The build-up of hazardous heavy metals in fish might potentially endanger the well-being of both fish consumers, particularly humans.

Khan (2021) conducted a study to explore the bioaccumulation patterns of chromium (Cr), nickel (Ni), cadmium (Cd), and lead (Pb) in the carnivorous fish species Armatus at various locations along three rivers in Malakand Division, Pakistan. The research also investigated the site-specific accumulation of these heavy metals in the cells of M. Armatus at one location in River Panjkora. The levels of chromium (Cr) in the fish muscles ranged from 10.2 ± 3.5 to 29.8 ± 17.3, nickel (Ni) 24.7 ± 13.1 to 104.5 ± 27.1, cadmium (Cd) 0.77 ± 0.17 to 2.4 ± 0.12, and lead (Pb) 7.5 ± 5.3 to 75.2 ± 41.0 milligrams per kilogram (mg/kg) of wet weight, respectively. In Armatus, the sequence of metal accumulation in various organs is as follows: kidneys exhibit the highest concentration, followed by the liver and skin, with lower deposition in muscles and gills. The bioaccumulation factor (BAF) readings of the metals in the fish muscles follow the order of Cr > Pb > Ni > Cd. The BAF results suggest that these metals are being stored in the fish tissues and could potentially pose a health risk to individuals who regularly consume them (Khan, 2021).

Paul et al. (2019) conducted a study on the quality of water, genetic toxicity, and histological alterations in the liver, gill, and kidney of the common carp, which, under exposure to lead nitrate. The fish were divided into 6 categories while subjected to varying concentrations of lead nitrate. Following 96 hours, samples of gill, liver, and kidney tissues were obtained and subsequently subjected to microscopic investigation. The water samples were examined following a 96-hour treatment period. The main changes seen under a microscope were swollen gills, damaged lamellae, liver cell degeneration, and larger spaces in renal capsule.

The research demonstrated a significant increase in the number of micronuclei with higher doses. Our findings indicate that the exposure of common carp to lead nitrate can result in genetic damage. Prolonged exposure to high levels of lead nitrate poses a potential threat to the health of fish. The outcomes suggest that lead nitrate not only adversely affected the *Cyprinus carpio* fish but also impacted the water quality (Paul et al., 2019).

Olalekan et al., (2015) researched to analyze the impact of water conditions in Lake Volta on various fish species. The study focused on fish that live near the bottom, in the middle, and near the surface of the lake. This research utilized water data and analyzed samples to determine its content. Fish were collected from four distinct locations: Oti River in Sabra, White Volta in Daboya, Black Volta in Bamboi, and Lower Volta in Amedeka. The study encompasses research conducted from February 1995 to January 1996, examining various numerical values and information. Among the locations, Black Volta and Oti River exhibited the highest amount of water in Lake Volta and the highest average annual rainfall. Test results revealed varying levels for conductivity, dissolved oxygen, pH, BOD, calcium, chloride, total alkalinity, hardness, magnesium, ammonia-N, phosphate, nitrate, nitrite, and sulfate. In the lake, 16 types of fish live near the sea floor, 6 types that swim in open water, and 11 types that live on the bottom of the lake. The research found that the water was good when compared to water in other lakes. The abundance of fish in the water indicated that human activities had minimal impact on its health (Olalekan, et al., 2015).

District Swat is well known for its beautiful natural scenery all over the world. People visit this place to engage in enjoyable and relaxing activities. Many people in the area rely on tourism and the sale of fish for their livelihood. The number of fish in the ocean is going down and there are many reasons for it. It is essential to determine the source of water pollution, develop strategies to safeguard fish and water and assess the current structure of our organizations based on this data. A study by Anwar, (2018) indicated that the primary sources of water pollution are waste from industries such as marble and cosmetics, as well as from hotels, auto workshops, and service stations. Additionally, household sewage, floods, landslides, diminished water supplies, and river mining were identified as contributing factors to water pollution. The research suggested ways to stop harmful ways of fishing and completely forbid catching fish during their breeding season. The rules about fishing and getting a license should be changed. Special places for fishing and mining should be set up to protect fish and lower water pollution. The hatcheries need to be bigger, and more trees need to be planted to deal with the floods. People must be educated on preventing water pollution, prohibiting unauthorized fishing, and disposing of trash properly. Water and fishing policies should be made that meet the specific needs of each area. Using it to make energy or natural fertilizer can minimize the problem of trash. The fisheries department requires additional staff and funding to increase their work capacity (Anwar, 2018).

Malik, et al., (2020) investigated the detrimental effects of water pollution on fish and their habitats. Many of our daily activities depend on the availability of freshwater, for drinking and other growing needs. The increase in pollution has led to a less stable environment, negatively impacting both the land and aquatic organisms. Toxins such as insecticides, herbicides, heavy metals, industrial waste, and oil were frequently deposited into the water, causing contamination and endangering the aquatic flora and fauna. The release of many pollutants into the water led to the rapid death of numerous aquatic creatures. Small amounts of pollutants being released cause the toxins to build up in the bodies of fish. Fish can be adversely affected by water pollution in two different manners. It can make them immediately sick (acute) or make them sick over time (chronic). This can weaken their immune system, slow down their metabolism, and damage their gills and skin. Pollution-induced diseases include tail rot, fin rot, gill disease, liver damage, and ulcers (Malik et al., 2020).

The occurrence of toxic metals is leading to aquatic pollution in freshwater lakes and rivers of Pakistan. The primary cause of pollution can be attributed to the release of contaminated water from urban areas, industrial facilities, and agricultural lands. The polluted water containing harmful metals had negative impacts on aquatic life and humans. Afzaal et al., (2022) Indicated that freshwater in Pakistan has experienced contamination from heavy metals. The levels of these metals in soil, water, and fish were scrutinized to explore their interconnectedness. The study also probed into the origins of these metals. It was observed that the existence of toxic metals in water and sediment can result in their accumulation in fish organs. The pollution of rivers like Chenab, Ravi, Indus, and Kabul, has been identified as causing adverse impacts on both aquatic life and individuals who consume the tainted water. The pollution of the River Ravi in Central Punjab is a result of the extensive waste it accumulates from both factories and cities. It is more polluted than other rivers in Pakistan. The River Indus was in a good state in Pakistan as it had plenty of water and a lower number of factories. People could eat freshwater fish from the Indus, Chenab, and Jhelum rivers. The presence of heavy metals has resulted in difficulties for society, and changes in the environment, and has affected the economy. It is crucial to purify the contaminated water before discharging it into rivers and streams to safeguard the well being of fish and humans from the harmful impact of metals (Afzaal et al., 2022).

1.6 Problem Statement

River water bodies' account for around 80 to 90% of the fish captures obtained from the inland fisheries in the Northern regions of Pakistan. To meet the increasing demands, it is imperative to prioritize the preservation of fish in sufficient numbers, while also ensuring their quality and variety. Surface water bodies are subject to many human-induced influences (Demeke & Tassew, 2016). The physicochemical properties have had a detrimental impact on the health of fish in that region. It is crucial to conduct a thorough assessment of how water quality parameters affect the fish in the Dasu region of the River Indus to ensure sustainable practices and informed decision-making in the area. This study specifically examines the physicochemical characteristics that affect the quality of water, as well as the toxicological effects on the fish population living in it (Olalekan, et al., 2015). The Dasu region of the Indus River has been significantly affected by the presence of different pollutants, which can be attributed to the establishment of hydropower dams, manufacturing, and intensive methods of agriculture. It is of utmost importance to evaluate the level of toxicity of water in this region and the potential consequences it may have on fish. The aim of the present study is to examine the physical and chemical attributes of water quality, along with the composition and distribution of the fish population in the area, in order to assess the implications.

1.6.1 Research Objectives

The main goal of this research is to analyze the physicochemical characteristics of water quality of Dasu region of Indus River, specifically near Dasu Hydropower Project DHPP, and understand the quality of water and their impact on the fish. The specific research objectives are as follows:

1. To assess the water quality by physicochemical parameters in the Indus River and its adjacent selected tributaries of Dasu region.
2. To evaluate the toxicity of water on fish in the study area.

CHAPTER 2

METHODOLOGY

The study aimed to evaluate water toxicity in the Dasu region of Indus River and understand its potential effects on fish. The research involved analyzing physicochemical variables and heavy metal level in water. Additionally, this study assessed heavy metal levels in fish organs and examined oxidative stress resulting from water pollution.

2.1 Study Area

The study focused on the Dasu region, encompassing both upstream and downstream areas of the Dasu Hydropower Project (DHPP). It specifically examined the river and adjoining tributaries in this defined region.

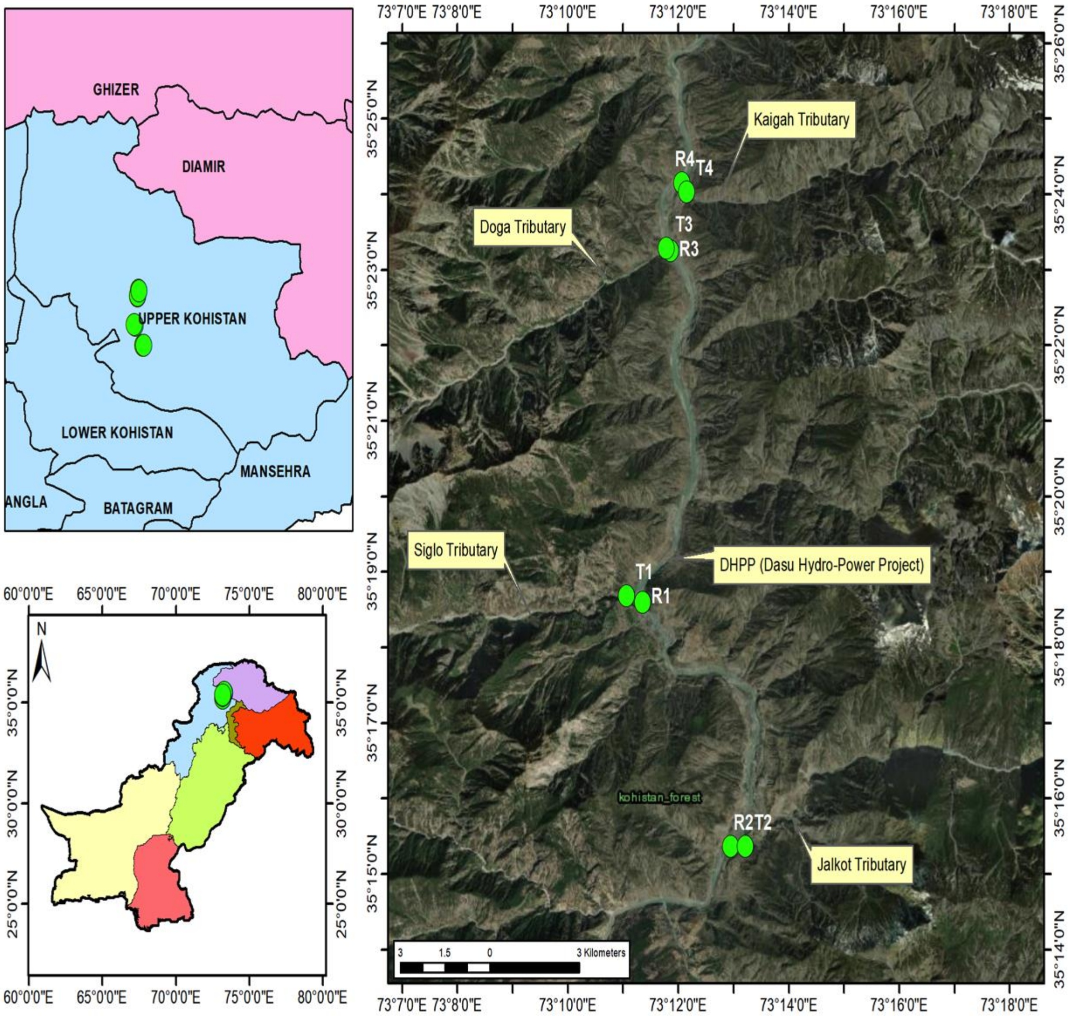

Figure 2.1. Study region map of Dasu Hydropower Project and adjacent tributaries.

2.2 Identification of Sampling Points

The study focused on various sampling sites within the Dasu region of the Indus River and its tributaries. The research covered eight sampling locations along the Indus River, both above and below the Dasu Hydro-Power Project (DHPP), and provided their global positioning system (GPS) coordinates in a summarized table.

Table 2.1 List of sampling points along with their corresponding GPS coordinates.

Sampling sites: No.	Sampling locations	GPS Coordinates
1	R1	35^{o}31’08” N 73^{o}18’80” E
2	R2	35^{o}15’22” N 73^{o}12’57” E
3	R3	35^{o}23’15” N 73^{o}11’52” E
4	R4	35^{o}24’09” N 73^{o}12’04” E
5	T1	35^{o}31’17” N 73^{o}18’62” E
6	T2	35^{o}15’22” N 73^{o}13’13” E
7	T3	35^{o}23’15” N 73^{o}11’48” E
8	T4	35^{o}24’7.8” N 73^{o}12’4.9” E

The first sampling point, R1, was strategically chosen on the left side downstream of the Dasu Hydropower Project (DHPP), representing a critical area below the dam. Similarly, the second point, R2, was selected on the right side downstream of DHPP. These points, R1 and R2, situated on both sides of the Indus River below the

DHPP, serve as primary sampling locations for assessing the toxicological impacts of water quality on fish in the Dasu region. Moving our emphasis upstream, on the left side of the Indus River, we have the third sampling point, R3, while upstream on the right side is the fourth point, R4. These locations, R3 and R4, serve as main sampling points for understanding the water quality and potential toxicological impacts on fish before reaching the DHPP. Moving to tributary points, the fifth sampling point, T1, is located on the left side, entering into the Indus River downstream of the DHPP. Similarly, the sixth point, T2, is positioned on the right side, contributing to the Indus River downstream of the DHPP. These tributary points, T1 and T2, serve as essential reference locations for assessing the impact of sidewise tributaries on water quality and toxicological impacts on fish. Finally, the seventh sampling point, T3, is situated on the left side, entering the Indus River upstream of the DHPP, while the eighth point, T4, is on the right side, contributing upstream. These tributary points, T3 and T4, are crucial reference locations for understanding the quality of water entering the Indus River from sidewise tributaries before reaching the DHPP at Dasu region.

2.2.1 Dasu Hydro-Power Project

The Dasu Hydropower Project is located within the Indus-Kohistan region, situated in the lower Himalayas and encompassing mountainous elevations ranging from 2,000 to 4,000 meters. There are even taller mountains, exceeding 5,000 meters, located at a distance. Within the project area, the Indus River traverses a deep valley in the mountain piedmont plain. Its entry into the impact area occurs from the west, near Diamer, where it flows through a relatively broad valley toward Shatial. The valley takes a southerly bend near Lootar, forming a narrow gorge-like structure. The region features small lateral tributaries and nullahs that discharge water originating from rainfall and snowmelt into the main river. At this specific juncture, the Indus River is characterized by a swift flow and carries a significant sediment load. The area maintains a low population density, hosting 34 small villages or hamlets within the direct impact zone, evenly distributed along both the left and right banks of the Indus. The largest town in the vicinity is Dasu – Komila, with an approximate population of 7,150 residents (Ahmed et al. 2023, WAPDA, 2014).

Regarding the physiography of the Dasu Hydropower Project area, it is marked by steep and rocky terrain, with limited expanses of level or gently sloping land.

Cultivation is primarily observed along certain nullahs, often on terraced soils or areas designated as alluvial fans and old river terraces.

In terms of geology, the project is situated in the "Kohistan Arc Complex zone," an area formed during the mid-Cretaceous period, characterized by a mix of igneous and sedimentary rocks. Positioned near the convergence point of the Asian and Indian continental plates, the region has experienced notable thrusting, uplifting, tilting, and plutonic activity. The dominant rock types in the project area are granulates and amphibolite's on both sides of the Indus. A significant geological fault, the Khoshe fault, plays a pivotal role in determining the layout of underground rock chambers housing powerhouses and influencing the design and construction of tailrace tunnels. The riverbed comprises a combination of glacio-fluvial deposits, terrace material, and landslide materials, with unconsolidated moraine (glacial) deposits found at higher elevations on the slopes. (WAPDA, 2014, Ahmad et al. 2018).

Table 2.2 Geographical features of Dasu Hydropower Project. (Ahmed et al. 2023)

Geological Feature	**Description**
Location	Dasu dam site is in Kohistan District, KPK, Pakistan, and its reservoir area extends into Gilgit Baltistan province.
Project Development Site	Along the Indus river, 7 km north of Dasu town, 348 km north of Islamabad
Dam Height	242 meters (gravity dam)
Hydropower Generation Capacity	Expected to generate 4320 MW hydropower.
Annual Rainfall	Average annual rainfall in Dasu area is 648-mm. Hottest month: July (max temperature of 36 ºC). Coldest month: January (min temperature of 2.5 ºC).
Geomorphology	Rugged terrain with high surface relief, ranging from 740 to 2600 meters on the left bank and 2800 meters on the right bank of the

	Indus river.
Topographic Feature	North–south trending mountain ranges along both sides of the Indus river.
Surrounding Geography	Surrounded by the higher Himalayas in the north and lower Himalayas to the south.
Himalayan Mountain System	Consists of four main ranges running from south to north in the Gilgit-Baltistan province, formerly northern areas of Pakistan. Prolonged Himalayas orogeny started with the collision of Indian and Eurasian plates around 60–65 million years ago. The NW drift of the Indian plate began around 130 million years ago.
Composition of Rocks	Mainly composed of norite/gabbro norite, granulite/meta diorite, and amphibolite from the upper to the lower reaches. Contact boundary between granulite and amphibolite named as the Khoshe contact, a normal type of contact with an inactive fault with little displacement
Rock Distribution	Granulite is exposed on both sides of the Indus river around the dam site. Amphibolite is distributed downstream of the dam site below the Khoshe fault, and norite/gabbro-norite rocks are highly distributed in the upper reach of the study area from the Sazin region up to the Diamer Basha dam site.
Total Length	570 m (1,870 ft.)

2.2.2 Tributaries

There are various tributaries downstream and upstream of DHPP. Some of them were small and low water flow while some having high flow of water and little big. For this reason two tributaries were selected downstream (T1 and T2) and two upstream (T3 and T4) as a reference points.

2.3 Collection of Fish Samples

In the present study, an edible fish species was selected as the experimental subject and collected from 4 points along the Indus River. I.e., R1 & R2 (downstream) and R3 & R4 (upstream) of DHPP including four tributaries T1 & T2 (downstream) while T3 & T4 (upstream) of DHPP. Fish species used for analysis was *Schizothorax* (Snow trout) genus of cyprinid family. The choice of this species for the study was based on its prevalence in the Dasu region than other species, As per Khan et al. (2018), Usman et al (2017); this particular genus is widespread in the Indus River and serves as a primary food source for the local residents, aligning with the outcomes of the current study.

Schizothorax (Snow Trout)

The *Schizothorax* is a fresh water fish of family *Cyprinidae* is a native fish of Dasu region. The *Schizothorax plagiostomus*, commonly known as Snow Barbel, is a fish species that is widely distributed in the Indus River including tributaries. In Pakistan, the Snow Barbel is a crucial food fish, especially in mountainous and sub-mountainous regions. As per a study, the Snow Barbel exhibits an algae feeding habit, mostly devouring algae adhering to rocks and pebbles consistently over the year. During summer, the Snow Barbel’s diet mainly consists of Spirogyra and Ulothrix, while their minimum amount occurred during autumn ("Wikipedia contributors," 2023).

Table 2.3 Scientific details of Schizothorax (*Cyprinidae*).

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Cypriniformes
Family	*Cyprinidae*
Species	*S. Plagiostomus*
Binomial name	*Schizothorax plagiostomus*
Common name	Common Carp, Snow trout, sawati fish

2.4 Experimental Design

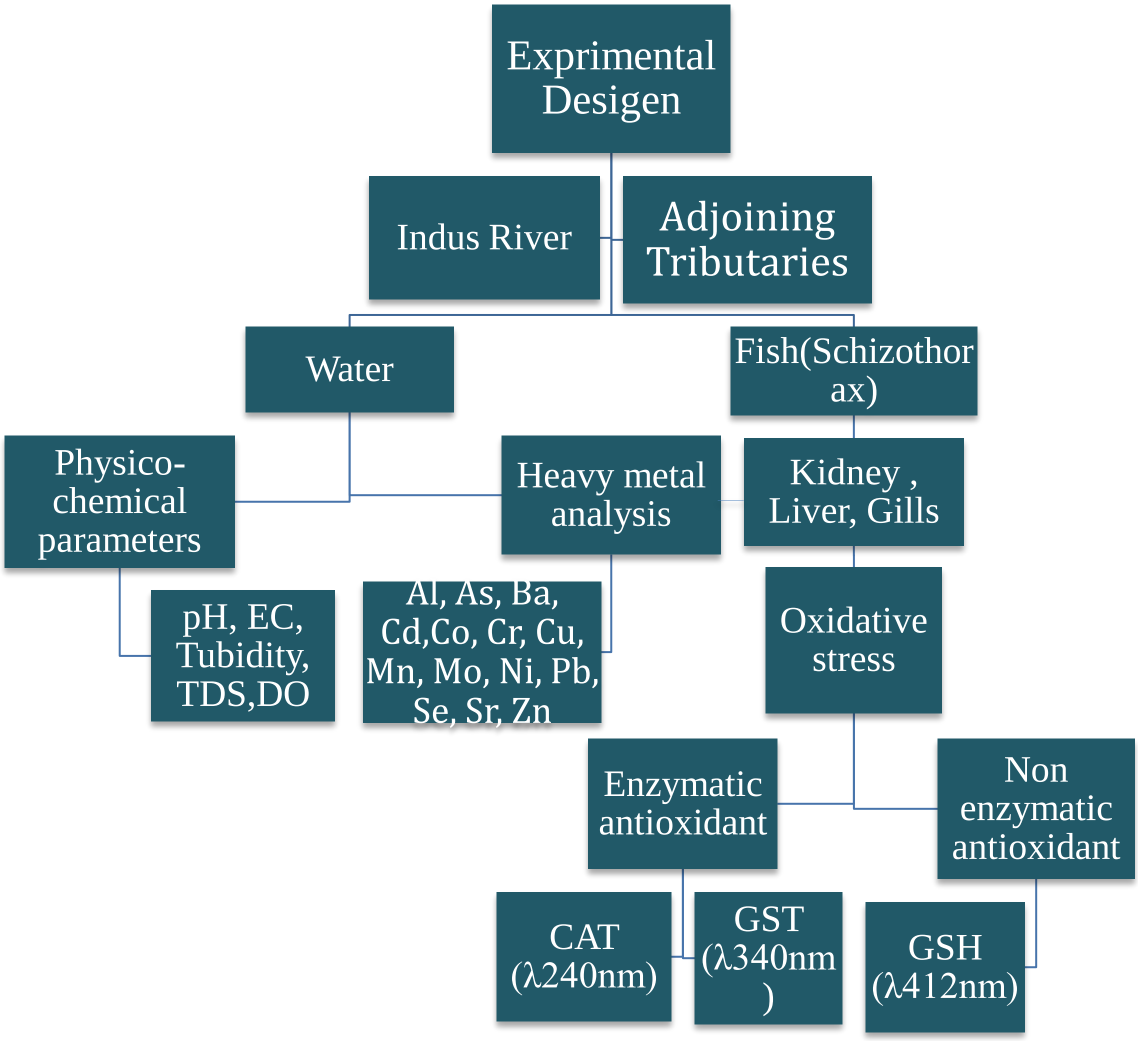

Figure 2.2. Schematic representation of the experimental layout utilized in the study.

2.5 Collection of Water Samples

Water samples were taken in duplicate from eight sites in the Dasu region of the Indus River, including upstream, downstream, and bordering tributaries. The samples were taken in clean, sterilized bottles and evaluated for pH, turbidity, total dissolved solids, and electrical conductivity using standard methods (APHA 2012).

2.6 Physicochemical Analysis of Water Samples

Water samples were collected in sterilized plastic bottles to ensure sterility during the physicochemical parameter testing. Each location provided duplicate samples. Physicochemical parameters such as pH, Dissolved oxygen (DO), electrical conductivity (EC), and total dissolved solids (TDS) were measured using a turbidity meter (TU-2016).

2.7 Analyzing Heavy Metals in Water Samples

Here are the procedures involved in analyzing heavy metals in water samples

2.7.1 Digestion Process of Collected Water Samples

Each sample was labeled based on its respective location, encompassing two sets from each of the eight distinct locations along the Indus River and adjacent tributaries (both downstream and upstream of DHPP). Following the transfer of 100ml of water from each sample into pre-cleaned plastic bottles using distilled water, 5ml of nitric acid (HNO3) was added to the mix, and it was left to stand overnight. Subsequently, the samples were filtered using Whatman paper no. 42. Ultimately, the treated samples were kept in a dark and dry environment until the analysis was conducted (APHA 2012).

2.8 Fish Samples

Snow trout, scientifically known as *Schizothorax plagiostomus*, were procured for the study. These freshwater fish exhibited an average length of 21cm and an average weight of 206g. The collection involved a total of 24 fish, with 12 sourced from various locations along the Indus River (both downstream and upstream of DHPP) and the remaining 12 from different points of adjoining tributaries. This collection took place in

May 2023, facilitated by skilled fishermen employing various fishing gears. Particular emphasis was placed on maintaining consistency in the size and weight of the gathered specimens. The fish were immediately placed in an oxygen-filled bag, preserved in an icebox, and promptly transported to the environmental science laboratory of Bahria University H-11 Campus Islamabad.

2.9 Analysis of Heavy Metals in Fish Organs

The steps involved in analyzing heavy metals in the organs of *Schizothorax plagiostomus* are as follows.

2.9.1 Chemicals

Hydrochloric acid (HCl), Nitric acid (HNO_3), and Formalin were employed in the experimental procedure. All of which were readily accessible in the Environmental Science laboratory of Bahria University H-11 campus, Islamabad.

2.9.2 Dissection and Sample Preparation

Fish dissection was carried out in accordance with the fish handling protocols established by the OECD guidelines (OECD, 1984). Upon arrival at the laboratory, the fish underwent stunning through the addition of 5% formalin in water, following the procedure described by Munir et al. (2016). Thorough washing with distilled water ensued, and measurements of weight and length were meticulously taken for each fish to ensure uniformity in size and guarantee the accuracy of results. Following this, cleaned tools from a dissection box were used in the dissection process. Organs, including liver, gills and kidneys, were collected in cleaned glass petri dishes. These organs were then washed with clean water and oven-dried at 120°C for 45 minutes. The subsequent step involved in preparing a homogenate by grinding the dried organ of fish samples into fine particles using a pestle and mortar. Lastly, the frozen homogenized samples were prepared for subsequent processing.

2.9.3 Digestion of Fish Samples

After thawing to room temperature, each sample, weighing one gram, was placed into beakers as per Munir et al.'s (2016) procedure. Following this, 10ml of aqua regia (HNO_3: HCl - 3:1) was put into each sample using a cylinder that was graduated. The samples were heated at 80°C for 5 minutes and subsequently raised to 120°C for an additional 15 minutes on a hot plate, ensuring thorough digestion of the organs in the aqua regia solution. After cooling to room temperature, each sample was adjusted to a volume of 35ml by adding 25ml of distilled water. The next step was to filter the samples with Whatman paper no. 42, collected the resultant filtrate in bottles made of plastic, and preserved them in a dry, dark space till study, as described by Ogamba et al. (2016) and Munir et al. (2016).

2.9.4 The ICP-OES Analysis

Laboratory preparation of water and fish samples, following standardized operating procedures, took place at environmental science laboratory of Bahria University in Islamabad. Inductively Coupled Plasma Optical Emission Spectroscopy (5110 ICP-OES) was employed for the analysis of As, Al, Ba, Cd, Co, Cu, Cr, Mn, Mo, Ni, Pb, Se, Zn and Sr, in both water and fish organ samples. The analysis was conducted at the National Water Quality Laboratory (NWQL) of the Pakistan Council of Research in Water Resources (PCRWR) in Islamabad. The recorded concentrations of heavy metals were expressed as mg/l for water samples and mg/kg for fish tissue samples.

2.9.5 The Statistical Analysis of Results

The statistical analysis of the data employed Microsoft Excel version 16.0. The data, characterized by statistical measures such as mean and standard deviation following the approach outlined by Naccari et al. (2015) and Fatima and Naeem (2016), was visually represented using graphs and tables. Furthermore, the results obtained for water and fish were compared with the established permissible standards.

2.10 Evaluation of Metal Contamination

Assessment of toxic metal accumulation involved the calculation of bioaccumulation factor/transfer factor and metal pollution index for fish organs. This approach aimed to provide a succinct understanding of the impact of metal concentrations on the organs.

2.10.1 Bioaccumulation Factor Calculation

The bioaccumulation factor (BAF) was calculated by dividing the amount of a given metal in fish tissue (expressed in mg kg-1 dry weight) by the amount in water (expressed in mg l-1) (Voigt et al., 2014; Monikh et al., 2015; Javed et al., 2016). The equation for BAF is as follows.

BAF = the amount of the same metal in water (mg/l) divided by amount of metal in dry fish tissue (mg/kg).

2.10.2 MPI (Metal Pollution Index)

In order to evaluate the site's quality, the Metal Pollution Index (MPI) for heavy metals was calculated following the method given by Usero et al. (1997). The MPI has been defined as the nth root of the multiplication of contamination factors for metals and is calculated using the formula listed below:

$$MPI = (Cfa \times Cfb \times ---------- Cfn)\ 1/n$$

Here, Cfa, Cfb, and Cfn denote the levels of metal n in the sample. (Hassaan et al., 2016 ; Javed et al., 2016). The contamination factor (CF) is calculated using the ratio of observed concentrations to the natural abundance of a certain metal. These CF values are classified into four levels for monitoring the enhancement of a single metal pollutant in water and fish during a certain time period (Ali et al., 2016; Voigt et al., 2014):

1. Low Level- (CF < 1)
2. Medium Level- (1 < CF < 3)
3. Considerable Level- (3 < CF < 6)
4. High Level- (CF > 6)

2.11 Analysis of CAT, GST and GSH Levels

Outlined below are the procedures for evaluating oxidative stress by analyzing the activities of CAT, GST and GSH, resulting from the level of heavy metals loads in the organs of *Schizothorax plagiostomus* (Snow trout).

2.11.1 Chemicals

In the well-equipped Environmental Science laboratory of Bahria University H-11 Campus Islamabad, a comprehensive array of essential chemicals was readily available for various scientific endeavors. Among the substances accessible were Sulfosalicylic acid, Ethylene diamine tetra-acetic acid, Sodium hydroxide, Hydrogen peroxide, Potassium dihydrogen- phosphate (KH2PO4), Dipotassium hydrogen phosphate (K2HPO4), 1-chloro, 2,4, dinitrobenzene (CDNB), and 2,5, di-thiobis tetra-nitro-benzoic acid (DTNB). This diverse collection of chemicals provided a robust foundation for conducting a wide range of experiments and analyses within the realm of environmental science, enhancing the research capabilities of the laboratory.

2.11.2 Dissection of Fish Samples

Following the OECD guidelines (1984) for fish handling protocols, the dissection process was carried out. Upon reaching the laboratory, the fish were accommodated in an aquarium glass box containing water from their native environment. Each fish was then individually euthanized and dissected using sterile dissecting tools. The gills, kidneys, and liver, three distinct organs, were extracted from each fish sample and meticulously arranged in cleaned glass petri dishes. Finally, every single organ underwent a thorough washing process with distilled water in preparation for subsequent procedures (Javed et al., 2016).

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2.11.3 Preparation and Extraction of Post-Mitochondrial Supernatant (PMS)

Preparation of post-mitochondrial supernatant procedure involved immersing precisely 2g of each organ of collected fish, including gills, kidneys, and liver, into a meticulously prepared 30ml buffer solution of phosphate (0.1 M, pH 7.4). This immersion was followed by a strategic freezing phase, maintained for duration of 24 hours to ensure optimal sample preservation. Subsequently, the samples underwent a carefully controlled thawing process, allowing them to return to room temperature, after which they were subjected to a thorough homogenization process employing a pestle and mortar. The resulting homogenate, representing a harmonious blend of the sampled tissues, was then methodically transferred into Eppendorf tubes for further processing. The next step involved subjecting the homogenate-filled tubes to a centrifugation process lasting 10 minutes, conducted at 1000rpm. This centrifugation step facilitated the separation of components within the homogenate, enabling the subsequent filtration process through Whatman paper no. 42 (Khalil et al., 2020). This specific filtration step played a crucial role in isolating nuclear debris from the homogenate, resulting in a clarified supernatant. Storing it in a designated freezer promptly preserved this supernatant, carefully devoid of nuclear debris. This meticulous preservation step ensured the integrity of the sample for subsequent biochemical analysis, aligning with the methodology outlined by Javed et al. (2016).

2.11.4 Analysis of Enzymatic Antioxidant Parameters

2.11.4.1 Analysis of Catalase (CAT) Activity

The determination of CAT activity followed the procedure was used by Khalil et al, (2020). A 3ml assay mixture was prepared for the analysis. To initiate the biochemical process, an initial step involved the meticulous creation of a reaction mixture. This was achieved by combining precisely 100 μl (0.1 ml) of the sample with 1.9 ml of a potassium phosphate buffer solution characterized by a concentration of 0.05M/50mM and a pH value of 7.0. Subsequently, the reaction was set into motion by introducing 1 ml of hydrogen peroxide (H2O2) at a concentration of 19mM. This carefully orchestrated procedure served as the foundation for the subsequent biochemical analyses, ensuring accuracy and reliability in the assessment of the sample's properties. We promptly took readings for duration of 3 minutes at 30-second intervals, starting immediately after the initiation of the reaction, and measured at 240nm. The quantification of enzyme activity was expressed in units per milligram of protein, utilizing a molar extinction coefficient of 3.94 millimoles per centimeter, as indicated in previous studies (Javed et al., 2016; Santos et al., 2004; Paul & Sengupta, 2013).

2.11.4.2 Analysis of GST Activity

To conduct the analysis, a 2ml assay mixture was meticulously prepared. The initial stage involved forming the reaction mixture by combining 1.8ml of buffer solution of potassium phosphate (0.1M/100mM, pH 6.4) with 0.1ml of 30mM 1-chloro 2,4 dinitrobenzene. Following this, 0.1ml of the sample was introduced into the mixture to initiate the reaction. Readings were recorded over a 3-minute duration at 30-second intervals, commencing immediately after the initiation of the reaction, and assessed at 340nm. The enzyme activity was quantified as units per milligram of protein, utilizing a molar extinction coefficient of 9.6 millimoles per centimeter (Farombi et al., 2007; Javed et al., 2016).

2.11.5 Analysis of Non- Enzymatic Antioxidant Parameters

2.11.5.1 Analysis of GSH Activity

To conduct the analysis, a 3ml assay mixture was meticulously prepared. Initially, equal volumes of 1ml homogenate sample and 1ml sulfosalicylic acid (4%) were combined and left to incubate at room temperature for an hour. After the incubation process, the samples were centrifuged at 3000 revolutions per minute for 15 minutes, obtaining a 0.5ml filtrate. The mixture was then treated with 2.3ml of a buffer composed of potassium phosphate (0.1M, pH 7.4), giving rise to a total assay volume of 2.8ml. To activate the reaction, add 0.2ml of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (5mM in 0.1M potassium phosphate buffering solution - a pH of 7.2 + 0.1mM EDTA). Observations have been taken at 412nm and recorded at every thirty seconds for 3 minutes. And lastly resulting enzyme activity is expressed in units/milligram of protein, with a molar extinction coefficient of 14.15 millimoles per centimeter (Santos et al., 2004; Javed et al., 2016; Pandey et al., 2003;).

2.11.6 UV Spectrophotometry for CAT, GST and GSH Reading

In accordance with established procedures, thorough preparations were undertaken for both fish and water samples at the Bahria University laboratory in Islamabad. The comprehensive evaluation of oxidative stress levels in fish encompassed the meticulous assessment of catalase (CAT), glutathione S-transferase (GST), and reduced glutathione (GSH) activities within the fish samples, was conducted by the UV4000 Spectrophotometer at Environmental Science laboratory H-11 Campus

Islamabad. Readings were recorded over a 3-minute period at 30-second intervals, specifically at wavelengths of 240nm, 340nm, and 412nm.

2.11.7 Protein Assay Estimation

Upon recording the readings, the determination of units per milligram of protein for CAT, GST, and GSH activities across all organs involved a three-step process.

In the preliminary step, the unit per milliliter of the enzyme was accurately calculated employing a specific formula tailored for this purpose. Subsequently, the second phase involved the utilization of Christian and Warburg's renowned 280-absorbance method (1942) to precisely ascertain the milligrams of protein per milliliter of the enzyme. Conclusively, in the third stage, the determination of units per milligram of protein was accomplished using the comprehensive total estimation protein. This multifaceted calculation ensures a comprehensive assessment of protein activity in the studied organs, maintaining accuracy and precision (Khalil et al., 2020).

- Unit/ml enzyme (nanomoles of enzymes present per gram of sample tissue) =

$$\frac{(\Delta OD/\text{min Sample} - \Delta OD/\text{min Blank})*1}{\text{Coefficient} * \frac{\text{total reaction volume}}{\text{volume of enzvme taken}}} \times \frac{\text{Total volume of enzyme extract}}{\text{Fresh weight of tissue (g)}} \times \text{Total protein} \times 1000$$

- Unit/mg Protein = $\frac{\text{Unit/ml enzyme}}{\text{mg protein/ml enzyme}}$

The 280 Absorbance Method

The 280-absorbance method developed by Christian and Warburg was employed for assessing the overall protein concentration in tissues. This method relies on measuring ultraviolet absorption at the wavelengths of 280nm to 260nm; this particular method of determining protein concentration relies on the existence of aromatic amino acids within proteins. What make this approach notably advantageous are not only its chemical simplicity but also its efficiency in accurately gauging protein concentration, preservation of samples without destruction, and rapid execution. The determination of concentration of protein is accomplished through the application of the provided formula;

Concentration of protein in milligram per milliliter = 1.55 × A280 -0.76 × A260.

Where 1.55 and 0.76 represent constants for protein contents used in the methodology developed by Christian and Warburg in 1942.

2.11.8 The Statistical Analysis of Results

The statistical analysis of enzymes was performed utilizing prism software (Graph-Pad 7.03), which is a dedicated statistical software platform. Each value was presented along with its standard error of the mean (SEM). To identify significant differences among the values of various organs of fish sample from river and tributaries, In the event of a significant difference being identified by ANOVA ($P < 0.05$). Furthermore, to evaluate noteworthy distinctions among river locations and tributaries, a one-way ANOVA was employed (Khalil et al., 2020).

CHAPTER 3

RESULTS AND DISCUSSION

Water, a fundamental component crucial for sustaining life on our planet, serving, as an effective solvent that upholds ionic balance and supplies vital nutrients (Jameel et al., 2012). However, recent human activities, particularly industrial and agricultural advancements, have resulted in the degradation of water quality, posing significant challenges to both the availability of safe drinking water and the well-being of aquatic ecosystems. This deterioration is accompanied by increasing worldwide apprehension regarding the discharge of heavy metals into freshwater sources, given the heightened toxicity of metals in aquatic organisms. Moreover, the enduring properties of these metals pose an ongoing risk to the sustainability and health of lakes, rivers, streams, and the diverse aquatic life within them (Xue et al., 2024). In response, the present study addresses this concern by evaluating quality of water, examining physical and chemical parameters, and assessing the toxic metal concentrations in water and in fish samples. So, the heavy metal bioaccumulation in the collected organs of fish and oxidative stress caused due to pollution of metals load were determined. The samples of fish and water were collected from various locations of Indus River and its nearby tributaries near the Dasu Hydropower Project (DHPP).

3.1 Physicochemical Analysis of Water Samples

The evaluation of water quality relies significantly on the physicochemical characteristics of water. In this research, a subset of these properties, like Turbidity, pH, EC, TDS, and DO, were assessed in samples collected from the Indus River and nearby tributaries in the vicinity of the Dasu Hydropower Project (DHPP).

In the course of this investigation, the results of parameters were subjected to a comparative analysis with the standards set by the World Health Organization (WHO, 2017). These standards provide permissible limits for turbidity, pH, electrical conductivity, and total dissolved solids. The limits of the parameters are as follow; turbidity >5 NTU, pH 6.5-8.5, EC 250µS/cm, TDS 1000 mg/L, respectively. It is important to highlight that the World Health Organization (WHO) has not established specific standards for DO in this context.

Table 3.1. Physicochemical features of water samples collected from various sites along the Indus River and nearby tributaries of study area.

Locations	pH	EC (µs/cm)	DO (%)	TDS (mg/l)	Turbidity (NTU)
R1	7.1	138.4	23.8	97.9	185
R2	6.83	117.5	29.9	83.5	93
R3	6.7	160.3	30.3	114	104
R4	6.8	163	26.2	116	243
T1	6.9	115.1	25.2	81.9	75
T2	7.1	34.1	27.2	24.4	22
T3	6.6	104.1	25.5	74.1	8.22
T4	6.9	47.5	30.3	33.9	17.7
WHO	6.5-8.5	250	_	1000	>5

All values were compared with WHO (2010) guidelines; blank cell indicate that no standards have yet been set by WHO.

The assessment of physical and chemical variables, comprising turbidity, pH, electrical conductivity (EC), total dissolved solids (TDS), and dissolved oxygen (DO), in collected water samples from several points along the Indus River and its adjacent tributaries near DHPP is detailed in Table 3.1. Among various parameters assessing water quality, pH stands out as a crucial indicator of contamination in aquatic systems. Its significance lies in its ability to impact water quality by inhibiting pathogen survival and facilitating the solubility of metals (Khan et al., 2015). The acidity or alkalinity of water is influenced by various elements, including soil constitution, volume, terrain, precipitation, mineral dissolution, and the origin of the water. Changes in pH result from the presence of dissolved carbon dioxide and natural acids like fulvic and humic acids, which are produced during the decomposition of plant material. The recommended pH range for potable water falls within 6.5 to 8.5. Throughout this investigation, the recorded pH levels at research sites 1 through 8 were found to be comfortably within the WHO-endorsed range of 6.5 to 8.5. Deviations from 7.1 and 6.6 range can lead to disruptions in the synthesis of vitamins and minerals, a salty taste below pH 6.5, and issues like eye irritation and skin disorders above pH 8.5. Extremely high pH levels exceeding 11 can result in gastrointestinal problems such as hyperacidity, ulcers, and a burning sensation. Maintaining pH within the specified range is essential for ensuring water safety and preventing adverse health effects (Sakthivadivel et al., 2020).

Total dissolved solids (TDS) represent a crucial water quality parameter as they significantly influence the taste and portability of water by measuring the concentration of solid materials in the water. In the current study, TDS levels were observed to range between 24.3 and 97.9 mg/L across all study sites (1-8), falling within permissible limits. The dissolving capability of water, encompassing a variety of inorganic and certain organic minerals or salts such as calcium, potassium, sodium, bicarbonates, magnesium, chlorides, and sulfates, results in the undesired dilution of taste and color. This dissolution process plays a significant role in altering the sensory qualities and visual appearance of water. Synthetic organic chemicals, even in small concentrations, can introduce objectionable tastes and odors (Chang, 2005). Higher TDS values in water indicate increased mineralization (Meride and Ayenew, 2016), potentially leading to laxative or constipation effects. While elevated TDS concentrations in groundwater are generally non-harmful, individuals with kidney and heart diseases may be affected, experiencing symptoms such as paralysis of the tongue, lips, face, irritability, dizziness, and disturbances in the central nervous system (Sasikaran et al., 2012).

The electrical conductivity levels observed in the current study fluctuated between 34.1 and 163 µS/cm, all-remaining within the acceptable range. Electrical conductivity serves as an indicator of water mineralization, and its levels fluctuate based on the concentration of dissolved salts, a process often influenced by temperature, affecting the dissolution of salts in water (Benrabah et al., 2016). Pure water, being a good insulator, does not effectively conduct electric current. The conductivity of water increases with higher ion concentrations, which are determined by the amount of dissolved solids present. Consequently, electrical conductivity measures the ionic processes within a solution, enabling its capacity to transmit current (Meride and Ayenew, 2016).

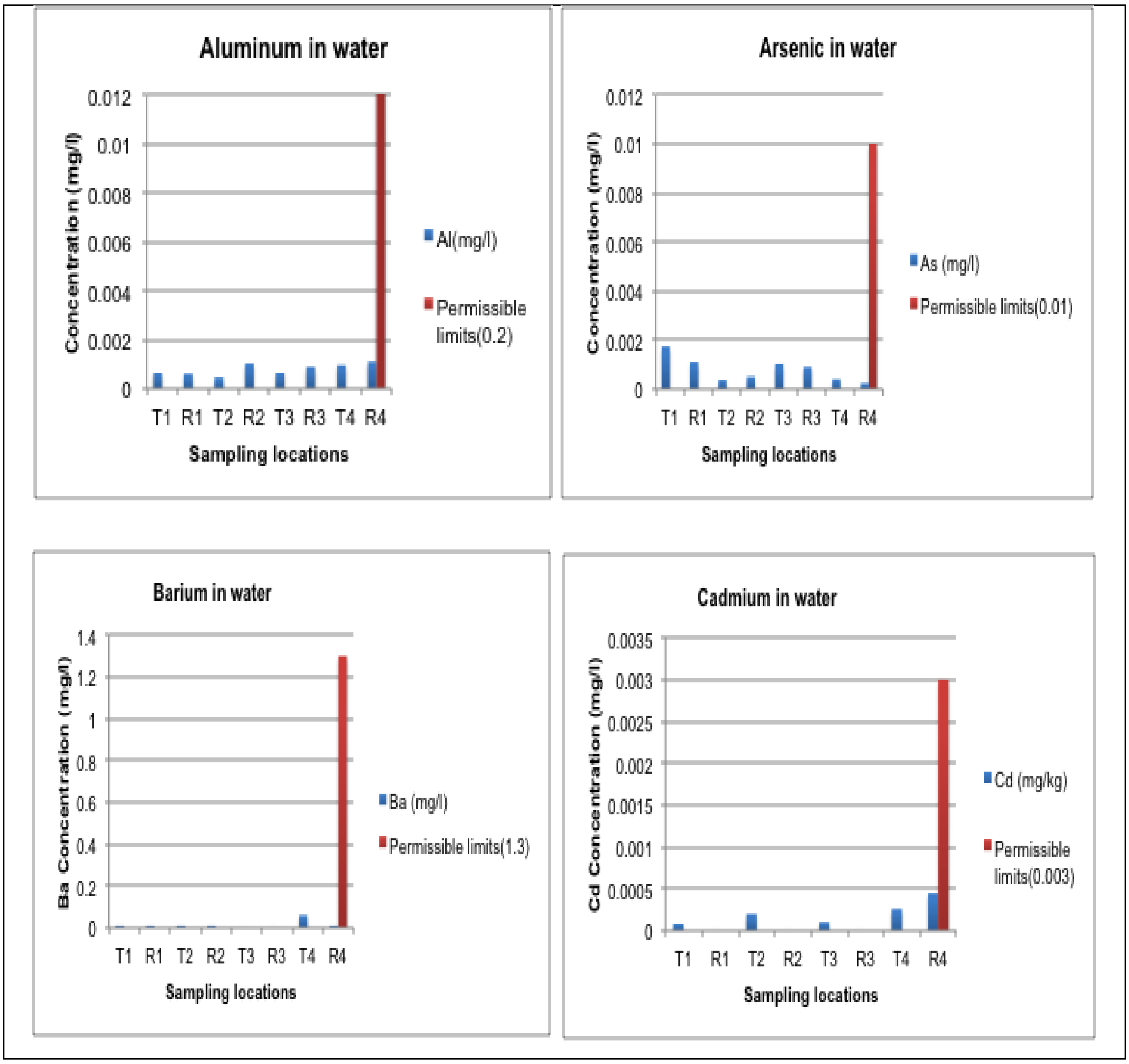
The turbidity levels in river water samples collected from various sites ranged from 93 to 243 NTU, While water samples from tributaries exhibited a turbidity range of 8.2 to 75 NTU, significantly surpassing the standard limits of >5 NTU, the heightened turbidity is attributed to suspended or dissolved particles in the water which scattering light. This leads to a hazy and opaque looks, potentially disrupting aquatic organisms by hindering sunlight penetration necessary for their well being (Khalil et al., 2020). Research indicates that the consumption of highly turbid water is associated with liver, thyroid, dermal, and ocular diseases, along with various impacts on immunological and reproductive systems (Kodavanti and Loganathan, 2017).

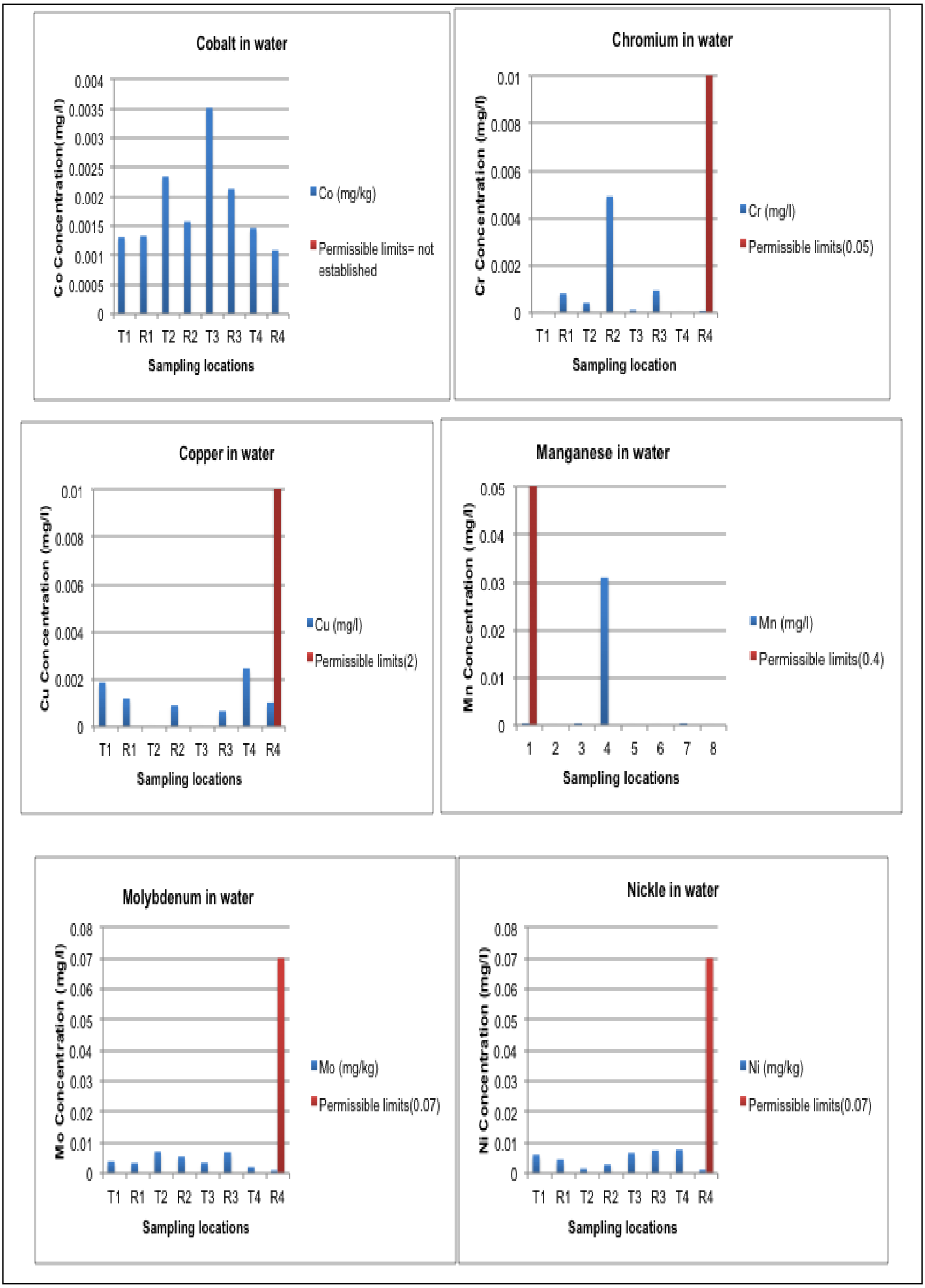
These parameters hold significant importance, as they constitute a crucial aspect of the environment, essential for the sustenance of life processes. Their significance is underscored by their vital role in maintaining the health of both fish and humans (Nazir et al., 2015). The assessment of physico-chemical properties is imperative, given their substantial impact on water quality, whereas the degradation of water quality can adversely affect aquatic life (Ali et al., 2016). In the study, the measurement of these variables establishes reference point for understanding pronounced impacts of toxic metals in water habitat and their impact on the fish.

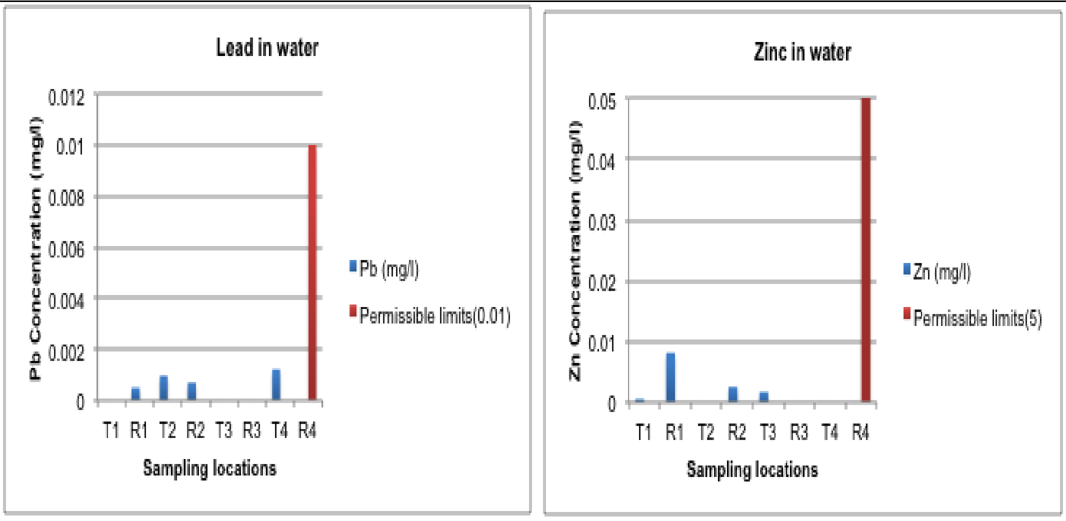
Consequently, the study broadened its investigation through evaluating the occurrence and concentrations of toxic metals in both samples of water and fish of the Indus River and adjacent tributaries near the Dasu Hydropower Project. Fish was selected as the focal point among biota due to its effectiveness as an indicator for the accumulation of metals loads in the overall aquatic ecosystem. Fish, acting as the principal and main source of pollutants, serves as a ubiquitous and commonly consumed edible product. This makes it a crucial indicator, especially considering possible negative consequences on human health following the consumption of contaminated fish. The study aims to shed light on the intricate dynamics of metal presence in water and fish, providing valuable insights for environmental management and human health considerations.

3.2 Metals in Water Samples

At eight distinct locations along the Indus River and its nearby tributaries, the concentrations of heavy metals, including aluminum (Al), arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), and zinc (Zn), were measured. The results are visually represented in Figure 3.1 and were subjected to a comparison with the established standards of WHO.




Cobalt in water
Co Concentration(mg/l)
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
T1 R1 T2 R2 T3 R3 T4 R4
Sampling locations
Co (mg/kg)
Permissible limits= not established
Chromium in water
Cr Concentration (mg/l)
0.01
0.008
0.006
0.004
0.002
0
T1 R1 T2 R2 T3 R3 T4 R4
Sampling location
Cr (mg/l)
Permissible limits(0.05)
Copper in water
Cu Concentration (mg/l)
0.01
0.008
0.006
0.004
0.002
0
T1 R1 T2 R2 T3 R3 T4 R4
Sampling locations
Cu (mg/l)
Permissible limits(2)
Manganese in water
Mn Concentration (mg/l)
0.05
0.04
0.03
0.02
0.01
0
1 2 3 4 5 6 7 8
Sampling locations
Mn (mg/l)
Permissible limits(0.4)
Molybdenum in water
Mo Concentration (mg/l)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
T1 R1 T2 R2 T3 R3 T4 R4
Sampling locations
Mo (mg/kg)
Permissible limits(0.07)
Nickle in water
Ni Concentration (mg/l)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
T1 R1 T2 R2 T3 R3 T4 R4
Sampling locations
Ni (mg/kg)
Permissible limits(0.07)



In Figure 3.1, heavy metal concentrations in water samples from the Indus River (R1-R4) and nearby tributaries (T1-T4) are graphically depicted

The concentrations of As, Co, Al, Ba, Cd, Cr, Cu, Mo, Mn, Ni, Pb, and Zn in water samples gathered from various points of the Indus River and its adjacent tributaries near DHPP are illustrated in Figure 3.1. Furthermore, the determined concentrations of these heavy metals in the water samples are well within the established permissible limits outlined by the World Health Organization. The findings indicate that the water quality, with respect to these specific heavy metals, meets the standards of World Health Organization for safe water consumption. The presence of toxic metals in collected samples of water from Indus River at Dasu Hydropower project were documented in the following sequence [River] = Mn > Mo > Ni > Co > Cr > Pb > As > Zn > Cd > Cu > Al > Ba. Whereas, in the adjacent Tributaries, the order of heavy metal concentration was water samples [Tributaries] = Ba > Mo > Ni >Co > Zn > Pb > As > Al > Mn > Cr > Cd. The comprehensive evaluation of heavy metal contamination in water samples from the study region concludes that the concentrations of all metals remain within the permissible limits set by WHO. This is a positive outcome, suggesting that the water in the Indus River and its adjacent tributaries near DHPP is safe for consumption, meeting international standards for water quality. The noteworthy revelation that all recorded heavy metal concentrations in the water samples adhered to established permissible limits of World Health Organization (WHO) is not only reassuring for human water consumption but also holds particular significance for the ecological health of aquatic life, particularly fish populations.

Previous studies have emphasized the diverse impacts and significance of heavy metals on aquatic environments. For instance, high concentrations of manganese (Mn) and molybdenum (Mo) have been associated with detrimental effects on fish health, affecting reproduction and growth (Liu et al., 2023, Wang et al., 2022). Mn has crucial role in metabolic regulation, acting as a co-factor for pyruvate carboxylase and superoxide dismutase, underscores its significance in maintaining biological homeostasis (Kumar et al., 2023). Nickel (Ni) and cobalt (Co) have been linked to bioaccumulation in aquatic organisms, posing long-term risks to the ecosystem (Blewett et al., 2016). The toxicological impacts of chromium (Cr) and lead (Pb) on fish health are well documented, with potential consequences for neurological and reproductive systems (Jamil Emon et al., 2023). Similarly, the study (Sayed et al., 2015) emphasized the notable accumulation of arsenic (As) in fish organs, especially the liver, leading to adverse effects on growth, hemato-biochemical parameters, and reproductive functions. Cadmium (Cd), characterized by its high toxicity and carcinogenic nature, ranks among the most hazardous agents in aquatic ecosystems (Jamil Emon et al., 2023), while several studies have consistently reported significant contamination of the aquatic environment with Cd, emphasizing cadmium's detrimental effects on fish physiology (Zhou et al., 2008, Olmedo et al., 2013). Copper (Cu) plays an important role in enzymatic processes and the production of hemoglobin, serving as a cofactor in various biological systems. However, an excessive intake of copper, surpassing permissible limits, can result in adverse health effects (USFDA, 1993). Al and Ba, are known to accumulate in aquatic organisms in freshwater, potentially causing harm to fish organs. Consuming contaminated fish poses health hazards to consumers. The study concludes that these heavy metals accumulate at different concentrations in various fish organs.

In the context of the Dasu region, where the hydropower project is altering the landscape, understanding the potential impacts on fish health is crucial for ensuring the sustainability of the aquatic ecosystem. As construction activities continue, the findings from this study become instrumental in shaping environmental policies and management strategies. The assessment of toxicological impacts on fish health provides a holistic perspective, enabling stakeholders to make informed decisions that balance the developmental needs of the region with the preservation of its ecological integrity. This multi-dimensional analysis, considering both water quality and its implications for fish health, contributes to a robust foundation for sustainable development practices around the Dasu Hydropower Project.

3.3 Assessment of Heavy Metal Levels in Fish Samples

The levels of various heavy metals, including Aluminum (Al), Arsenic (As), Barium (Ba), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), Lead (Pb), and Zinc (Zn), were investigated in fish specimens collected from eight different sites along the Indus River and its neighboring tributaries near DHPP. The findings, depicted in Figure 3.2, were then compared with the benchmarks established by the World Health Organization.

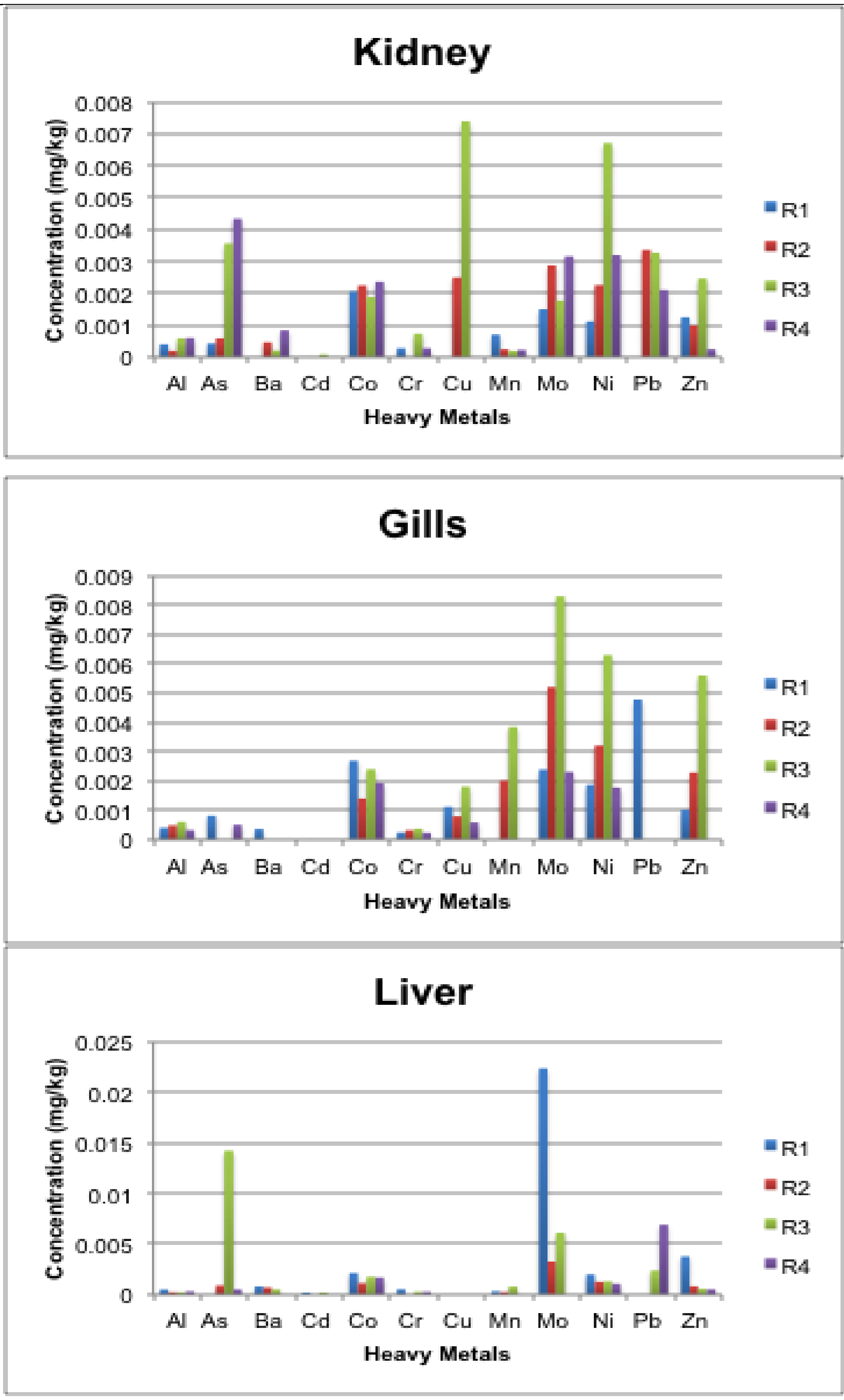


Figure 3.2 presents an analysis of heavy metal concentrations in fish tissues collected from various locations along the Indus River within the study area.

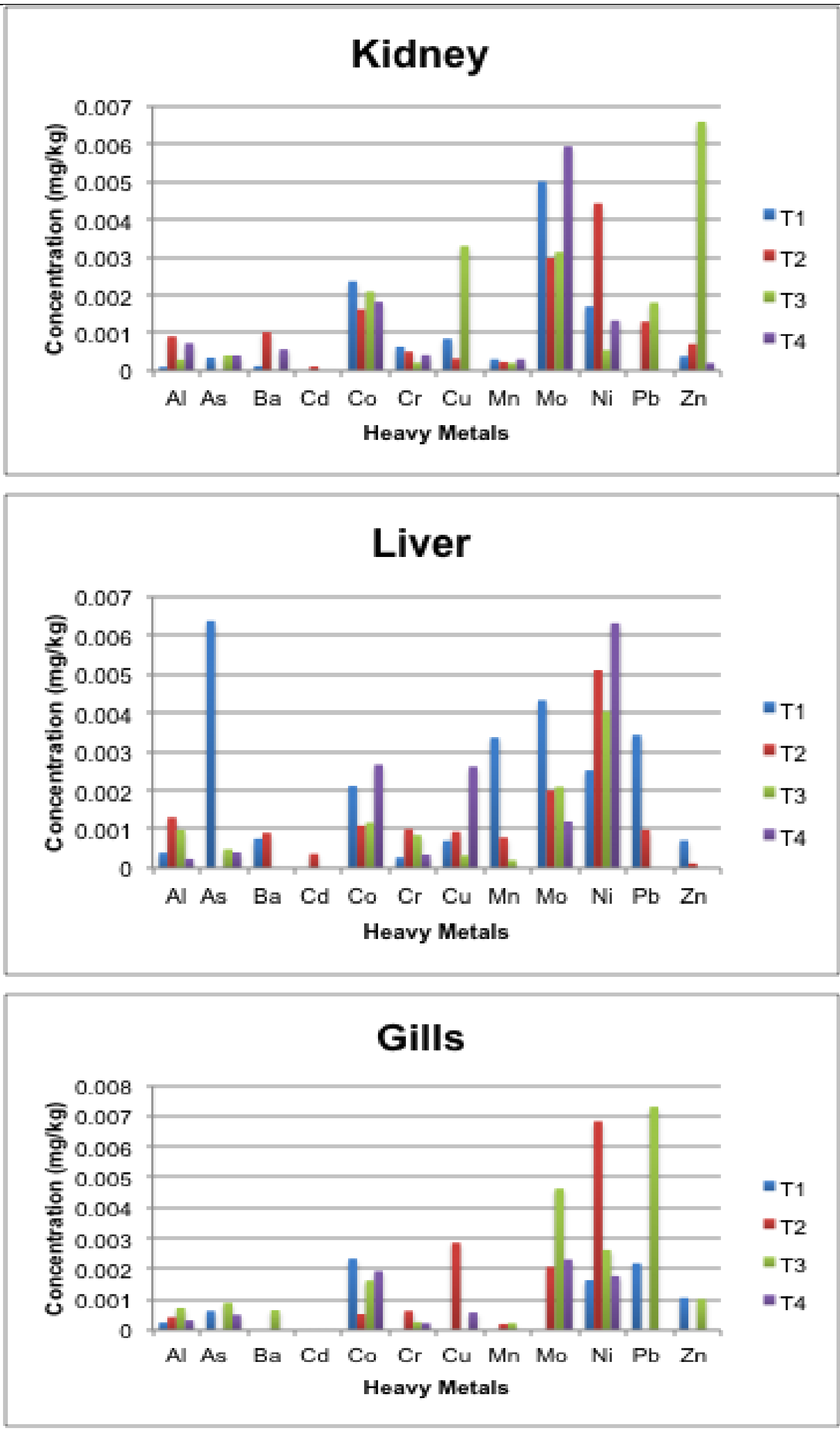


Figure 3.3 presents an analysis of heavy metal concentrations in fish tissues collected from adjacent tributaries of Indus River within the study area.

As shown in Figure 3.2 and 3.3, the mean concentration of Molybdenum (Mo) was most elevated in the fish from the river (0.005383mg/kg) and in tributaries fish (0.00324mg/kg), except T1-Liver and R4-Liver Mo was BDL (below detection limit). While Cd has the lowest concentration in river fish (0.000235mg/kg) and in tributaries fish (0.000107mg/kg), and was below detection limit in almost all sampling sites of river and tributaries except T2 (Kidney & Liver) and R3 (Kidney & Liver).

Average Ni concentration in fish organs ranged 0.003231mg/kg in tributaries fish while 0.002668mg/kg in river fish that fell within the permissible limit (WHO, 0.04mg/kg) according to (Fu & Wang, 2011; Munir et al., 2016). Pb was completely below detection limit in T4 site while in other sites of tributary and river it was detected of average concentration ranged 0.0038mg/kg in river fish organs and 0.002832mg/kg in tributaries fish organs. As also found below detection limits in R1 liver and gills of R3 and R2 as well as completely bellow detection limit in all organs (kidney, liver & gills) at Sampling site T2. The average concentration of Arsenic was ranged 0.002873 in river fish organs and 0.001156 in tributaries fish organs. The detected average concentration of Arsenic was within the permissible limit (WHO, 0.26mg/kg). Cobalt detected in all sampling sites with average range of 0.001971 in river fish organs and 0.001778mg/kg in tributaries fish organs.

Copper was detected in all sampling sites almost while in some organs it was below detection limit. The average concentration range of Cu in river fish organs and tributaries organs are 0.002365, 0.001383 mg/kg. Zn was detected in all organs of river fish except R4-gills, and some tributaries organs of samples. The average detected range of zinc in river fish organs are, 0.001779 and in tributaries fish organs are, 0.001346 mg/kg. The average concentration of Mn in river and in tributaries fish organs are, 0.000861mg/kg, 0.000644mg/kg. While in R4 liver and gills and T4 liver and gills as well as T1 gills manganese was below detection limit.

Aluminum was detected in all sampling sites of river and tributaries. The average concentration of aluminum in river fish organs and in tributaries fish organs were ranged 0.000405 and 0.000552 mg/kg. Chromium was only below detection limit in R2 (Kidney & Liver) and T1 (Gills). The average detected value of chromium in river and tributaries fish organs were 0.000352 and 0.000485 mg/kg. The results of the this research reveal that, in river fish, the standard sequence of heavy metal concentrations in the kidney is observed as Ni > Cu > Mo > As > Pb > Co > Zn > Al > Ba > Mn > Cr > Cd. In the liver, the order is Mo > As > Pb > Al > Ba > Co > Zn > Mn > Cr > Ni > Cd > Cu, while in the gills, it is Mo > Ni > Co > Cu > Mn > Pb > As > Zn > Ba > Al > Cr. In contrast, among tributaries fish samples, the pattern of heavy metal concentration in the kidney is Mo > Ni > Co > Zn > Cu > Pb > Al > Cr > Ba > As > Mn > Cd, in the liver, it is Ni > Mo > As > Co > Cu > Mn > Pb > Al > Cr > Ba > Zn > Cd, and in the gills, it is Ni > Pb > Mo > Co > Cu > Zn > As > Al > Cr > Mn. The current study's findings provide valuable insights into the distribution and bioaccumulation of toxic heavy metals in river fish and adjacent tributaries, offering implications for environmental monitoring and human health. The observed patterns in heavy metal concentrations across various organs and fish populations reflect the intricate dynamics of metal uptake, accumulation, and physiological interactions.

In this study, the prevalent presence of Nickel (Ni) in the kidneys of river fish emphasizes the bioavailability and uptake of this metal. Multiple studies have underscored Ni bioaccumulation in fish tissues, highlighting its potential sources and environmental implications (Palermo et al., 2015; Blewett et al., 2016). Additionally, nickel toxicity disrupts ion regulation and induces oxidative stress in fish (Leonard & Wood, 2013; Blewett & Leonard, 2017). Elevated levels of Copper (Cu) and Molybdenum (Mo) further contribute to the complexity of metal interactions, necessitating further exploration of the specific pathways and mechanisms involved in their accumulation. The liver, a key detoxification organ, exhibits a distinct order with Molybdenum (Mo) taking precedence, indicating its role in detoxifying and sequestering heavy metals. The prominence of Mo in the liver aligns with existing literature discussing its involvement in metals metabolism and detoxification processes in fish (Al-Ghanim et al., 2019; Yang et. al 2016). The sequence of heavy metals in the gills raises questions about potential implications for respiratory and ion-regulatory functions, underscoring the need for targeted studies on the physiological responses of gills to heavy metal exposure.

Tributaries fish samples reveal distinct patterns, indicating the influence of local environmental conditions on metal concentrations. The predominance of Molybdenum (Mo) in the kidneys of tributaries fish aligns with studies linking Mo bioaccumulation to specific environmental factors, such as sediment composition and water chemistry (e.g., Xu et al., 2016; Sun et al., 2020). Liver and gill patterns suggest a nuanced response to metal exposure in tributaries, emphasizing the importance of considering ecosystem-specific factors in understanding metal bioaccumulation dynamics.

To fully comprehend the implications of these findings, future research should delve into the ecological consequences of heavy metal bioaccumulation in fish and explore potential risks to human health through the consumption of contaminated fish. Additionally, incorporating spatial and temporal variations, alongside specific environmental parameters, will enhance the applicability of these results in environmental management and conservation efforts.

3.4 Bioaccumulation Factors

Bioaccumulation is the process of accumulation of different substances and elements in living tissue from the surrounding. To quantify the accumulation of heavy metal a ratio was established by comparing the metal levels in tissues with their corresponding environment (Monikh et al., 2015). Table 1 and table 2 describe the bioaccumulation factor of selected organs of fish samples from river and tributaries respectively.

Table 3.2 provides an overview of bioaccumulation factors observed in river samples across different organs.

Location	Organs	Al	As	Ba	Cd	Co	Cr	Cu	Mn	Mo	Ni	Pb	Se	Sr	Zn
R1	Kidney	0.65	0.39	BDL	BDL	1.55	0.34	BDL	BDL	0.45	0.25	BDL	BDL	BDL	0.15
	Liver	0.81	BDL	4.1	BDL	1.59	0.65	BDL	BDL	6.75	0.44	BDL	BDL	BDL	0.46
	Gills	0.65	0.75	1.8	BDL	2.02	0.29	0.95	BDL	0.72	0.42	9.5	BDL	BDL	0.13
R2	Kidney	0.2	1.18	2.35	BDL	1.43	BDL	2.78	0.01	0.53	0.8	4.8	BDL	BDL	0.39
	Liver	0.23	1.8	3.55	BDL	0.71	BDL	BDL	0.01	0.6	0.44	BDL	BDL	BDL	0.32
	Gills	0.47	BDL	BDL	BDL	0.89	0.07	0.89	0.07	0.96	1.13	BDL	BDL	BDL	0.89
R3	Kidney	0.66	0.66	BDL	BDL	0.89	0.78	11.3	BDL	0.26	0.9	BDL	BDL	BDL	BDL
	Liver	0.25	15.7	BDL	BDL	0.83	0.29	BDL	BDL	0.9	0.18	BDL	BDL	BDL	BDL
	Gills	0.67	BDL	BDL	BDL	1.12	0.4	BDL	BDL	1.22	0.85	BDL	BDL	BDL	BDL
R4	Kidney	0.55	18.8	1.2	BDL	2.19	0.4	BDL	BDL	3.51	2.93	BDL	BDL	BDL	BDL
	Liver	0.28	2.22	BDL	BDL	1.56	3.86	BDL	BDL	BDL	0.97	BDL	BDL	BDL	BDL
	Gills	0.29	2.17	BDL	BDL	1.79	3.28	0.58	BDL	2.57	1.62	BDL	BDL	BDL	BDL

In sample R1, the BAF for Aluminum in the liver is 0.81, indicative of the concentration of Aluminum in the liver tissue is 0.81 times higher than in the surrounding water. Similarly, in sample R2, the BAF for Arsenic in the gills is 0.47, suggesting that the gills contain 0.47 times the concentration of Arsenic compared to the water.

Similarly, in T1 group of organs samples Mn was found in the highest concentration levels in liver. Besides this all the ratios were closely related to each other, Cd, Se, Sr were not detected in any of the samples suggesting that no source of contamination for these metals is not found in river and tributaries. Arsenic was found in T1, T2, and T4 samples are an indication of geogenic provenance.

Organs follow (liver>gills>kidney) trend, which shows that liver was most vulnerable to bioaccumulation relative gills and kidneys. The metal distribution exhibited the following trend in all organs: Mo > Cd > Pb > Sr > As > Cr > Se > Ba > Al > Cu > Mn > Zn > Co > Ni. This shows that Mo was the most while Ni was least accumulative element in the fish organs. It was found by (Javed & Usmani, 2015) that liver and gills of fish accumulate relatively higher quantities of metals from the surrounding water.

Table 3.3 Bioaccumulation factors of in different fish organs collected from tributaries

Location	Organs	Al	As	Ba	Cd	Co	Cr	Cu	Mn	Mo	Ni	Pb	Se	Sr	Zn
T1	Kidney	0.15	0.2	0.22	BDL	1.8	BDL	0.45	1.3	1.31	0.29	BDL	BDL	BDL	0.68
	Liver	0.6	3.66	1.39	BDL	1.61	BDL	0.38	14.7	1.13	0.42	BDL	BDL	BDL	1.27
	Gills	0.38	0.36	BDL	BDL	1.79	BDL	BDL	BDL	BDL	0.37	BDL	BDL	BDL	1.89
T2	Kidney	2	BDL	1.7	0.55	0.69	1.19	BDL	0.92	0.42	3.11	1.34	BDL	BDL	BDL
	Liver	2.89	BDL	1.5	1.8	0.47	2.38	BDL	3.12	0.28	3.58	1.01	BDL	BDL	BDL
	Gills	0.93	BDL	BDL	BDL	0.22	1.5	BDL	0.8	0.3	4.81	BDL	BDL	BDL	BDL
T3	Kidney	0.45	39	BDL	BDL	0.06	1.9	BDL	BDL	0.91	0.08	BDL	BDL	BDL	3.87
	Liver	1.51	0.47	BDL	BDL	0.33	7.63	BDL	BDL	0.61	0.62	BDL	BDL	BDL	BDL
	Gills	1.11	0.87	BDL	BDL	0.46	2.45	BDL	BDL	1.35	0.4	BDL	BDL	BDL	0.6
T4	Kidney	0.75	1	0.01	BDL	1.25	BDL	BDL	1.2	3.04	0.17	BDL	BDL	BDL	BDL
	Liver	0.24	1	BDL	BDL	1.82	BDL	1.07	BDL	0.62	0.81	BDL	BDL	BDL	BDL
	Gills	0.33	1.25	BDL	BDL	0	BDL	0.24	BDL	1.18	0.23	BDL	BDL	BDL	BDL

Presence and bioaccumulation of these metals in could have implications both for aquatic ecosystem and humans if they consumed water and fish from such contaminated sources. Additionally, presence of hazardous elements such as Arsenic, and lead suggest significant environmental contamination emphasizing monitoring and investigation of the aquatic ecosystems. Arsenic is highly toxic and has the potential to accumulate in various organs of aquatic animals and human connected food chain. It also magnifies in higher levels of food chain. It is ubiquitous because of its natural occurrence in the earth crust (Singh et al., 2015).

Over all the above results suggests that fish consumption from such contaminated sources can be detrimental to health of humans and other aquatic organisms.

3.5 Metal Pollution Index

Metal pollution index for every sample and three organs kidney, liver and gills were calculated to quantify total metals in one single value.

Table 3.4 presents Metal Pollution Index (MPI) values and the corresponding organ pollution status.

Sample Code	**MPI**	**Status**	**Sample Code**	**MPI**	**Status**
T1- KIDNEY	4.94	Considerable degree	R1- KIDNEY	1.82	Moderate degree
T1- LIVER	5.97	Considerable degree	R1- LIVER	2.19	Moderate degree
T1- GILLS	2.31	Moderate degree	R1- GILLS	1.49	Moderate degree
T2- KIDNEY	6.42	High degree	R2- KIDNEY	1.69	Moderate degree
T2- LIVER	1.09	Moderate degree	R2- LIVER	1.72	Moderate degree
T2- GILLS	1.59	Moderate degree	R2- GILLS	1.66	Moderate degree
T3- KIDNEY	6.71	High degree	R3- KIDNEY	2.33	Moderate degree
T3- LIVER	3.12	Considerable degree	R3- LIVER	1.04	Moderate degree
T3- GILLS	3.79	Considerable degree	R3- GILLS	1.34	Moderate degree
T4- KIDNEY	6.33	High degree	R4- KIDNEY	1.68	Moderate degree
T4- LIVER	8.14	High degree	R4- LIVER	3.9	Considerable degree
T4- GILLS	2.41	Moderate degree	R4- GILLS	2.41	Moderate degree

Pollution level or degree was found out according to the criteria given by (dos

Santos et al., 2016). The grading system classifies the degree of pollution based on the metal pollution index values. It includes categories such as "Moderate degree," "Considerable degree," and "High degree," likely representing the severity of pollution.

Table 3.4 explains that tributaries T1 to T4 generally exhibit higher degree of pollution as compared to river samples R1, R2, R3 and R4 an indication of higher levels of pollution in tributaries. The kidneys of fish from tributaries (T1, T2, T3, T4) constantly show high or considerable degrees of pollution, representing a substantial accumulation of pollutants, especially metals, in this organ. The livers of fish from tributaries and rivers also show varying degrees of pollution, with some samples exhibiting considerable or high pollution levels. The gills normally show lower pollution levels compared to the kidneys and livers, with majority samples characterized as having a moderate degree of pollution. Sample T4 exhibited high MPI values for both kidney and liver, describes potentially severe pollution in this tributary sample.

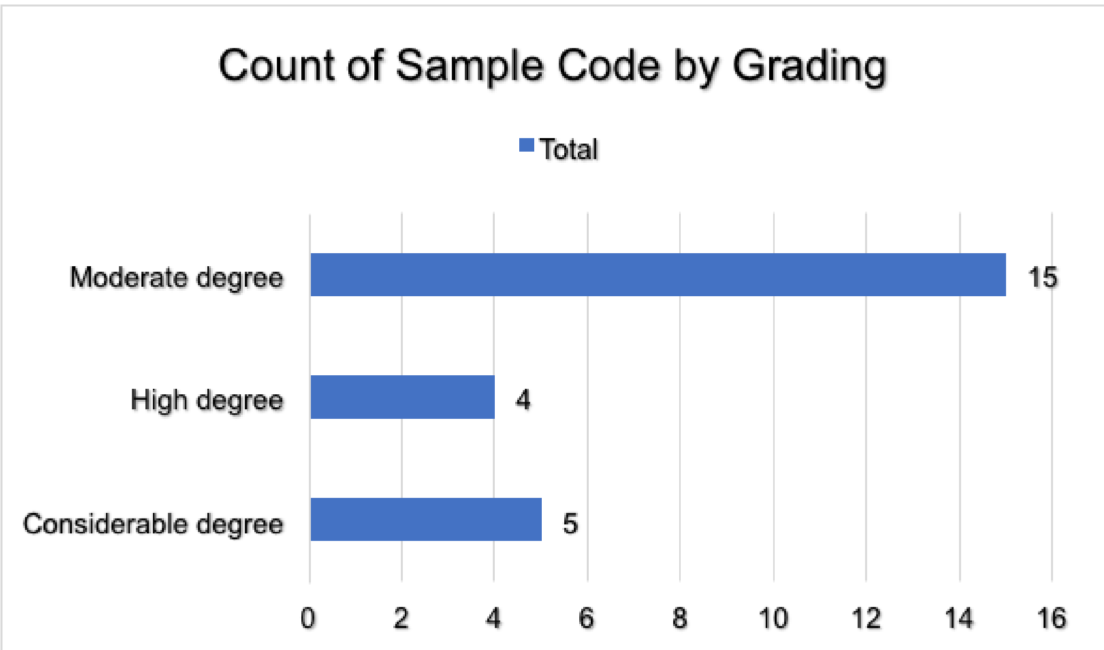


Figure 3.4 showcases the distribution of sample grades represented by counts.

Gills uptake salt ions and metals and is route to the body of fish for different substance. Nutrients and uptakes are then distributed to different organs. It is the case for toxins (Jovičić et al., 2015). The liver is considered a tissue with the highest potential for metal accumulation, given its high activity in the uptake and storage of heavy metals. It plays a vital role in both protein synthesis and detoxification processes, making it an essential organ in the overall metabolism and health maintenance of an organism. The liver tissue of fish exhibits a significant increase in metallothionein induction, indicating its crucial role in safeguarding against metal toxicity. Metallothioneins are proteins that bind to metals, reducing their harmful effects. As a result, liver serves as the primary organ directly exposed to toxicants in the fish, playing a defensive function towards exposure of metals. Additionally, the liver stores metals and plays a vital role in regulating their levels within the organism (Siraj et al., 2016). Kidneys also crucial to detoxification and filtration of toxin from blood stream, most of the ions and metals can be separate during urine formation but some tissue of kidney accumulate metals and stay there for long time in the kidney causes damage to its tissues, The MPI results conclude that liver and kidneys of the fish were loaded with more metals concentration as compared to gills and other organs.

3.6 Oxidative Stress Analysis

In the context of cellular physiology, oxidative stress arises when there is an imbalance between the generation of reactive oxygen species (ROS) and the biological system's ability to counteract or neutralize these reactive intermediates. This delicate equilibrium is crucial for maintaining cellular homeostasis, and any disruption can lead to oxidative damage to biomolecules and cellular structures. The consequences of oxidative stress extend beyond immediate cellular effects, impacting various physiological processes and contributing to the development of pathological conditions. Therefore, understanding and mitigating oxidative stress are essential aspects of ensuring overall cellular and organismal wellbeing''. Succinct explanation is the disparity between oxidants and antioxidants, which could potentially result in damage. However, all definitions of oxidative stress invariably address three key issues.

Fish undergo oxidative stress as a consequence of the generation of oxidizing agents, particularly Reactive Oxygen Species (ROS). Additionally, there is a potential for toxicity in their environment. Fish have evolved a mechanism and system for detoxification and reduction of ROS. When a large number of toxins enter the bodies of fish from the surrounding water, they can induce oxidative stress by inhibiting antioxidant enzymes and increasing ROS levels (Preiser, 2012). While ROS play a crucial role in proper cell function, their uncontrolled presence can be damaging. Antioxidant proteins and enzyme defense systems in fish work to keep ROS under control, ensuring the maintenance of cellular health and functionality.

Oxidative stress results in excessive ROS, which inactivates enzymes such as CAT, GSH, and GST etc. and damage tissue (Hu et al., 2021). CAT, GSH and GST are defense against oxidative stress since they work as enzymatic antioxidant and non-enzymatic antioxidant proteins. Hence, the current research study aimed to investigate the function of antioxidant enzymes such as Catalase, Glutathione and reduced Glutathione S-transferase against the oxidative damage induced in fish organs by heavy metals in surroundings water i.e., rivers, and tributaries. In the present study enzymatic and protein activities in unit per milligram protein were calculated for kidneys, liver and gills.

3.6.1 Detoxification and Antioxidant Enzyme Activities

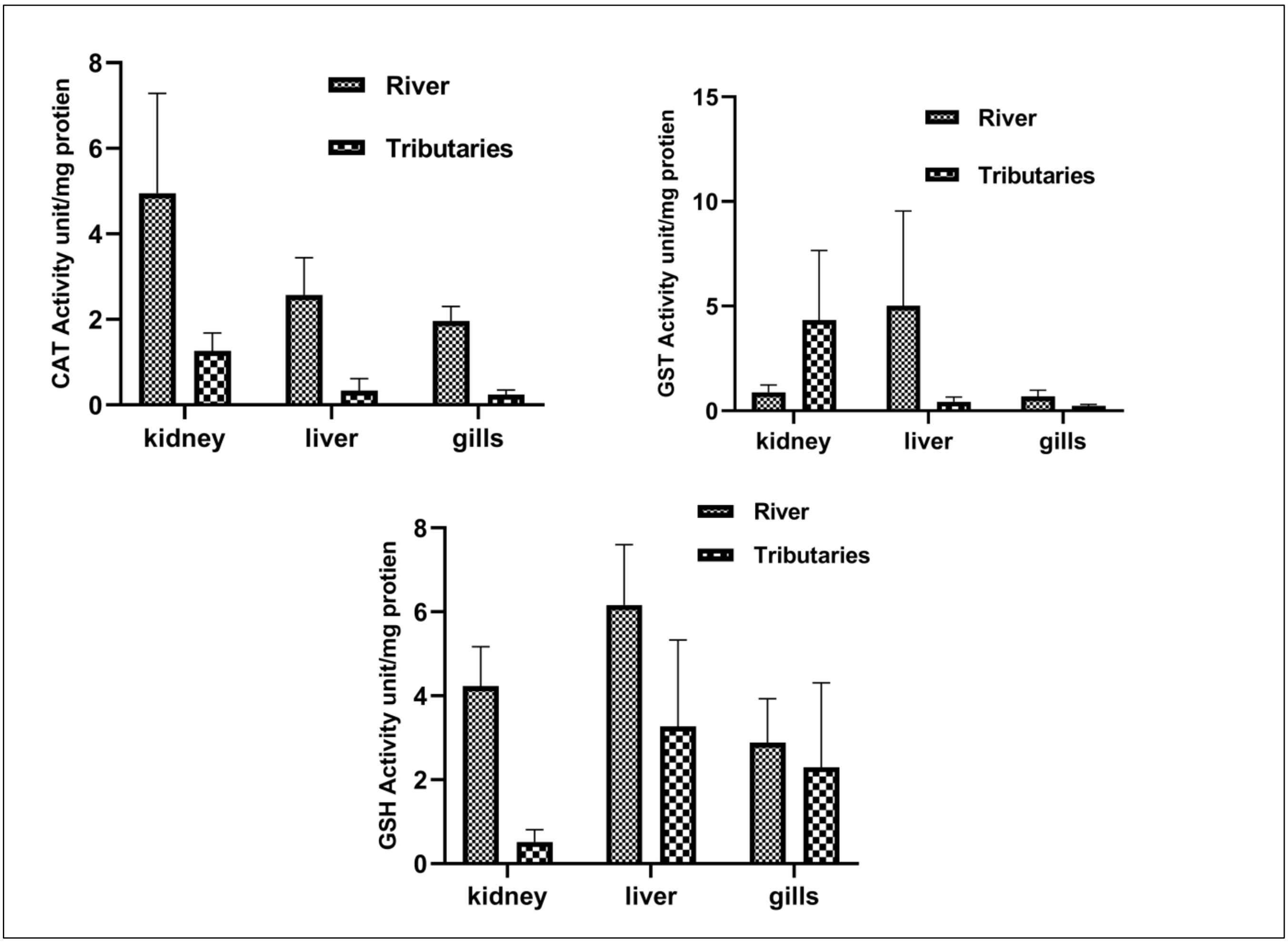


Figure 3.5 depicts the catalase (CAT), glutathione S-transferase (GST), and glutathione (GSH) activity levels observed in the kidney, liver, and gills of fish sampled from rivers and tributaries within the study area.

3.6.1.1 CAT Activity in Kidney Liver and Gills

As it is evident from figure 3.5 that catalase activity in the fish samples of tributaries far higher than the river fish samples. Kidney and liver have shown more activities than gills. It can be correlated that higher MPI and BAF of heavy metals for these samples and same organs have shown higher CAT activity per unit milligram protein. Actually, the activity of SOD, CAT, and GPOX enzymes depends on trace elements in such order copper/manganese/zinc, iron, and selenium. The rate-limiting factor for the activity of antioxidant enzymes is frequently the bioavailability of these trace elements (Storey, 1996). Since water was contaminated with some of these elements therefore enzyme CAT depicted such higher activities. Catalase plays a crucial role in converting excess hydrogen peroxide into non-damaging oxygen and water molecules (Nandi et al., 2019). Catalase helps maintain a balance of reactive oxygen species for the oxidative metabolism of sulfamethoxazole. It was also confirmed by (Vigneshkumar et al., 2013) that exposure of catalase to Lead causes an Increase in catalase activity. In kidneys the total activity of catalase increases which is induced by heavy metals in rates (Popova & Popov, 1998).

3.6.1.2 GST Activity in Kidney Liver and Gills

GST has shown relatively higher activity in the liver of river fish samples and kidneys of fish from tributaries while there is very low activity in gills. Since liver and kidney have more potential to accumulate metals the activity of GST was also high in these organs but contrast comes in tributaries and river samples. Liver accumulated more metal while in tributaries kidney accumulated more metals this may be adaptation to the different ecosystem and presence of different kinds of metals. Higher activities in these organs suggest oxidative stress. Following CAT and alkyl hydroperoxide reductase (AHR), the secondary line of antioxidant defense involves glutathione metabolism. This enzyme, Glutathione S-transferase (GST), facilitates the metabolization of reduced glutathione (GSH) through a conjugation reaction with nucleophilic xenobiotics or cellular components damaged by reactive oxygen species. This process results in the detoxification of these substances. The GSH substrate is reloaded for glutathione peroxidase (GPOX) and GST by NADPH-dependent glutathione reductase, utilizing oxidized glutathione (GSSG).

3.6.1.3 GSH Activity in Kidneys, Liver and Gills

Higher activities of GSH were observed in both river fish liver and kidneys. In contrast GSH activity was higher in tributaries fish liver and low kidneys. River samples contain more toxic elements, which induced oxidative stress more than metals in tributaries. But the increased antioxidant activity is in indication of induced oxidative stress.

GSH is an abundant soluble antioxidant present in different organs of living organisms. It is a tripeptide molecule comprised of glycine, cystine and glutamate and is synthesized in every vital organ in the body especially liver since liver is factory for most of the antioxidants in the body (Mytilineou et al., 2002). Glutathione can deliver spontaneously or with the help of peroxidase the hydrogen H for reduction of radicals. The Active group found in glutathione is thiol -SH group that performs the antioxidant activity. During this activity it is oxidized to cystine or cystine disulfide (Bounous & Molson, 2003)(Nie et al., 2013).

The increased activities and mechanisms of action of these enzymes indicate that heavy metals induce oxidative stress, both activating and inhibiting antioxidants such as enzymes like CAT and polypeptides like GSH in various organs. The kidney retains blood for an extended duration, while fish gills serve as storage sites for accumulating various salts, displaying comparatively heightened activities in these organs.

CONCLUSIONS

The study findings provide insightful conclusions regarding the environmental conditions and biochemical responses of fish in the study area. Firstly, it was observed that the water quality parameters within the study area generally met permissible limits, with the exception of turbidity levels in all water samples.

Secondly, the assessment of Metal Pollution Index (MPI) revealed significant metal accumulation in the liver (MPI: 8.14) and kidney (MPI: 6.71) of fish sourced from various locations along the Indus River and its tributaries, indicating potential risks associated with heavy metal pollution.

Moreover, the enzymatic analysis indicated notable Catalase (CAT) activity in fish samples from river, suggesting a heightened level of oxidative stress in these environments. Similarly, Glutathione S-transferase (GST) activity was observed to be higher in the liver of tributary fish and in the kidney of river fish, reflecting varied responses to detoxification processes between different locations.

Lastly, the study highlighted elevated Glutathione (GSH) activity in the liver and kidneys of fish collected directly from the river, underscoring the organism's adaptive mechanisms to counteract oxidative stress induced by environmental factors.

RECOMMENDATIONS

1. A study can be carried out on the monitoring of other chemical pollutants which may toxic to aquatic life should be designed.
2. Detail study including more sampling sites from the river and tributaries can be done.
3. Toxicological impacts can be studies on other aquatic species living in the environment.
4. People of the study area should be given awareness regarding the pollution and toxic effects on aquatic life and humans.

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APPENDICES

APPENDIX A

Sample Collection

Fish sampling in Indus River and nearby tributaries near Dasu Hydropower Project.

APPENDIX B

Sample Dissection and Preparation

The process involves dissecting the fish, extracting organs, and grinding dried organs post oven-drying

APPENDIX C
Digestion

Digestion process of extracted fish organs

Digestion

Soaking organs in a solution, followed by freezing, crushing, centrifuging, and filtering to produce the final extract

APPENDIX D

Solution Preparation and Analysis

Preparation of Solution and Analysis using ICP-OES and UV Spectrophotometer

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