Toxicological Evaluation of Heavy Metals Bioaccumulation and Oxidative Stress Biomarkers in Freshwater Fish *Channa Species*

Thesis

Submitted for the award of

Degree of Doctor of Philosophy

Zoology

By

Bharti Gupta

Enrollment No. MUIT0120038038

Under the Supervision of

Dr. Ramakant

Assistant Professor

Department of Zoology

Under the Maharishi School of Science and Humanities

Maharishi University of Information Technology

Sitapur Road, P.O. Maharishi Vidya Mandir
Lucknow, 226013

Session: 2020-21

July, 2024

Declaration by the Scholar

I hereby declare that the work presented in this thesis entitled **"Toxicological Evaluation of Heavy Metals Bioaccumulation and Oxidative Stress Biomarkers in Freshwater Fish *Channa Species*"** in fulfillment of the requirements for the award of Degree of **"Doctor of Philosophy**" submitted in the **Maharishi School of Science and Humanities**, Maharishi University of Information Technology, Lucknow is an authentic record of my own research work carried out under the supervision of **Dr. Ramakant**, I also declare that the work embodied in the present thesis-

i) is my original work and has not been copied from any journal/ thesis/ book; and

ii) has not been submitted by me for any other Degree or Diploma of any University/ Institution.

Signature of the Scholar

Maharishi University of Information Technology, Lucknow

Supervisor's Certificate

This is to certify that Ms. **Bharti Gupta** has completed the necessary academic turn and the swirl presented by him/her is a faithful record is a bonafide original work under my guidance and supervision. He/She has worked on the topic **"Toxicological Evaluation of Heavy Metals Bioaccumulation and Oxidative Stress Biomarkers in Freshwater Fish *Channa Species*"** under the School of Science, Maharishi University of Information Technology, Lucknow.

Date: **Name of Supervisor**

ACKNOWLEDGEMENT

With limitless humanity, I bow my head towards the almighty, merciful; compassionate, and supreme power of **'GOD'** who showed his mercy on me and blessed me to complete my Ph.D. successfully hitherto and present the piece of work for which I am eternally indebted.

I bow my head in great reverence to the omnipotent and omnipresent natural power **"Lord Hanuman Ji"** for their kindness and true blessings towards me.

I immensely rejoice to owe my gratitude to **Prof.** (**Dr.) Bhanu Pratap Singh**, Hon'ble Vice-Chancellor, **Dr. Vinod Kumar Singh,** Dean, College of Science, **Dr. Ramakant**, Department of Zoology for providing all sorts of facilities and amenities required during my study and research work.

I feel a golden opportunity and proud privilege to work under the talented and inspiring guidance, imperative and facile suggestion, untiring supervision, persistent encouragement, unending zeal, conspicuous ability, and constructive criticism of my Advisor, **Dr. Ramakant,** Assistant Professor, Department of Zoology, Maharishi University of Information Technology, I am extremely indebted to him for being meticulous throughout the period of investigation and preparation of this manuscript.

I am highly thankful to the Members of My Advisory Committee Late **Prof. Madhulika Singh and Dr. Sneha Verma** for their valuable guidance and thoughtful suggestions in the need of time during this research work.

Big thanks to **Dr. P. K. Saini** from ANDUA&T, Kumarganj, Ayodhya (Uttar Pradesh) for helping thesis and research article writing.

Words are inadequate to express the love and gratitude to my grandfather Late Shri Jagjeevan Prasad Gupta, Late Smt Santi Devi.

My parents **Shri Ramkumar Gupta**, **Smt Sandhya Gupta**, Bhaiya and Bhabhi, **Mrs. Sunita** w/o **Mr. Rahul**, **Mrs. Arpita** w/o **Mr. Mukul,** and My lovely Bhatiji, **Angel Gupta**, and family members for their constant encouragement and inspiration during my study.

It is more than likely that I have missed this acknowledgment and hope that those concerned although unnamed, will accept my thanks. I still solicit their benediction to proceed at every step of a perfect destined life.

Date: / / 2024

Place: Lucknow **(Bharti Gupta)**

ABSTRACT

The investigation entitled **"Toxicological Evaluation of Heavy Metals Bioaccumulation and Oxidative Stress Biomarkers in Freshwater Fish *Channa Species*"** was research works conducted at Department of Zoology, Maharishi University of Information Technology, Lucknow during 2022-23 and 2023-24. Water samples from Lucknow city area will be analyzed for pH, TSS, TDS, conductivity, and bacteria counts using Hanna H8921 model and flame photometry. Heavy metals concentration in water and fish samples will be assessed using ICPMS at ITRC Lucknow after liver tissue extraction and digestion. The study involved dissecting fishes, removing liver and muscle, homogenizing the homogenate, and analyzing lipid peroxidation, reduced glutathione, superoxide dismutase, glutathione-S-transferase, and catalase activity. The CAT activity was measured using UV-VIS spectrophotometer. The results provide valuable insights into the fish's health. The study involves analyzing liver samples from fish exposure groups, extracting RNA, removing genomic DNA contamination, and quantifying purity. RNA samples are analyzed using qRT-PCR and reverse transcribed, with primer pairs used for assessing heavy metal toxic genomic effects. Heavy metal contamination in aquatic ecosystems poses a significant threat to human health through the consumption of contaminated fish.

In North India, where freshwater bodies serve as crucial sources of sustenance, assessing the bioaccumulation of heavy metals and their impact on oxidative stress biomarkers in edible fish species is imperative for ensuring food safety and environmental health. This study aimed to evaluate the levels of heavy metals, including lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As), in commonly consumed fish species from North Indian freshwater systems and to investigate the associated oxidative stress biomarkers. A total of ten different fish species were collected from various freshwater bodies across North India, including rivers, lakes, and reservoirs. Samples were analyzed for heavy metal concentrations using atomic absorption spectrophotometry (AAS) after appropriate digestion procedures. Additionally, oxidative stress biomarkers such as lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were assessed in fish tissues to elucidate the potential oxidative damage induced by heavy metal exposure. This study found that Lead (Pb) exposure to fish *C. punctatus* resulted in toxicity, including haematological alterations, organ abnormalities, oxidative stress, and lipid peroxidation. The

study found that neither the control group nor the treatment group experienced significant differences in temperature or pH. The dissolved oxygen levels in the water decreased over time, and all fish groups displayed uncoordinated movement. The findings suggest that discharges of Lead into aquatic environments may pose a risk to animal wellbeing and aquatic life. The release of HMs into freshwater ecosystems may increase the risk of human exposure through food and drinking supplies. There is a pressing need for HM regulation and nanotoxicology data to assess exposures and dangers related to HMs. The food and feed industry is predicted to be significantly impacted by heavy metals products in the future.

Contents

LIST OF FIGURES

LISTOFTABLES

LIST OF ABBREVIATIONS

Abbreviation	Description
Pb	Lead
H_2O	Water
Zn	Zinc
HM	heavy metal
Cd	Cadmium
As	Arsenic
Al	Aluminums
DO	Dissolved Oxygen
TDS	Total Dissolved Solids
BOD	Biological Oxygen Demand
Mg^{++}	Magnesium
Ca^{++}	Calcium
MCHC	Mean Cell Hemoglobin Concentration
NBT	Nitroblue Tetrazolium
XOD	Xanthine-oxidase
ppm	Part Per Million
RBC	Red Blood Cell
Hb	Hemoglobin
Hct	Hematocrit
MCV	Mean Cell Volume
MCH	Mean Cell Hemoglobin
WBC	White Blood Cells
SOD	Superoxide Dismutase
O_2	Oxygen
S	Sinusoids,
PCV	Patchy Central vain
MMC	Melano-macrophage centers

DHV	Degeneration of hepatic veins,
DS	Dilated sinusoids
CCV	Congestion of the central vein,
A	Aneurysm
EI	Erythrocyte infiltration
LI	Lymphocytic infiltration
PF	Partial fusion
PLT and SLT	Secondary and primary lamellae are thick
CAT	Catalase
ITRC	Industrial Toxicology Research Centre
TSS	Total Suspended Solids
VU-VIS	Ultraviolet-Visible-Spectroscopy
DNA	Do-oxy Ribonucleic Acid
RNA	Ribonucleic Acid
qRT-PCR	Quantitative Real time-Polymerase Chain Reaction
HMs	Heavy Metals

LIST OF SYMBOLS

Symbol	Nomenclature
@	At sign
%	Percentage
( )	Parentheses
[]	Square Bracket
{}	Curly Bracket
&	And/Ampersand
/	Slash
-	Hyphen
.	Full stop
:	Colon
;	Semicolon
“ ”	Quotation Mark
‘	Apostrophe
?	Question Mark
****	Backslash

Chapter-1

INTRODUCTION

Water is a naturally occurring substance that is transparent, tasteless, odorless, and nearly colorless. It is the main component of the hydrosphere of the Earth and the bodily fluids of all known organisms. (Water is one of the basic needs for all living things. The survival of humans and other organisms depend on plants, and plants need good-quality water to flourish properly. Therefore, the quality of water is crucial for all living things, including humans, plants, and animals (Dos *et al.,* 2024). The constant deterioration of water bodies due to various anthropogenic activities has a negative impact on its intended use (Chawla, *et al.,* 2024). For instance, the degradation of most aquatic ecosystems might result from the discharge of wastewater that has not been properly treated into natural waterways (Xiong, *et al.,* 2024). Additionally, the use of contaminated water for irrigation, bathing, cleaning, or drinking may lead to several water-borne diseases.

1.1 Heavy metal contamination of water bodies

The toxicity, bioaccumulation potential, and widespread distribution of heavy metals make them crucial environmental pollutants. The Metals that are found in nature contamination include volcanic eruptions and the deterioration of rocks that contain metal while various industrial and agricultural activities and mining are the anthropogenic sources of heavy metals (Jagaba, *et al.,* 2024). Discharge of municipal sewage and industrial effluents is responsible for the inclusion of heavy metals in soil and water (Jadaa, W. 2024). In India, the main cause of water body pollution is the disposal of municipal sewage. It was observed that Class I and Class II cities generate approximately 29129 MLD (million litres a day) sewage which is further disposed of in water bodies (Sahoo *et al.,* 2024). Heavy metals are widely used in industries, and this will also affect how they appear in sewage (Buzukashvili *et al.,* 2024). Effluent from industries, towns, and urban areas also carries good amount of heavy metals. The majority of these elements eventually gather in the sediment of the soil and water bodies.

Even in minute amounts, heavy metals present in water sources can cause severe health issues for people and other organisms (Budi *et al.,* 2024). Apart from this, it has been discovered that human excrement contains heavy metals. The concentration of copper in feces is roughly 1.47 mg/kg, followed by lead, nickel, and cadmium (Ajala *et al.,* 2024). Bathing, tooth brushing, and hair washing are other ways that metals are discharged into sewers in residential settings as heavy metals are included in many household items, including medications, cosmetics, and polishes. Tin and fluoride are two common ingredients in toothpaste (Abedi *et al.,* 2024). Each year, the USA receives about 1,000,000 kg of tin from various sources. There have also been found that some anti-dandruff shampoos contain up to 1% selenium sulphide (Godse, G., & Godse, K. 2024). While the dose, mode of being exposed, chemical type, gender, age, genetics, and food intake of individuals exposed all affect the level of toxicity of heavy metals. Cadmium, lead, mercury, arsenic, and belong to the most important metals for the public's safety because of their severe toxicity (Godse, G., & Godse, K. 2024). These metallic elements are known to harm multiple organs even at low exposure levels, making them systemic toxicants (Singh, A., & Kostova, I. 2024). Furthermore, they have been classified as human carcinogens (known or probable) by the US International Agency for Research on Cancer (Kay, *et al.,* 2024).

1.2 Lead contamination

Among all heavy metals Lead (Pb) is a toxic substance that is not required for life, despite its higher level of cytotoxicity and lack of known biological functions (Harshitha, *et al.,* 2024). The majority of Pb in the environment is anticipated to come from mine, coal-fired power plants, ore smelting, batteries, industrial wastes, pesticides, and fuel additives (Adnan *et al.,* 2024). Owing to its widespread, not biodegradable and enduring characteristics, it leads to significant health and ecological problems necessitating appropriate cleanup techniques. Weathering and erosion occurrence for as much as 750,000 tones/year of zinc and lead inputs to environment according to Trivedy, A., & Khatun, M. (2024). Numerous studies that have been published have shown that lead has harmful impacts on both children and adults. These studies have demonstrated a link between low blood levels, reduced intelligence, reduced IQ, linguistic and speech deficits, delays or damaged neurobehavioral growth, reduced hearing acuity, retarded growth, diminished

attention span, and aggressive and conscientious behavior in kids **(**MacKinnon, D. F., & Chen, L. N. 2024).). High levels of lead intake in adults have been connected to reproductive effects in males and females, such as decreased sperm counts and unexpected miscarriages **(**Rotimi, D. E., & Singh, S. K. 2024). Acute lead exposure results in various organ failure like digestive issues, renal damage, severe brain injury, while chronic exposure may adversely impact the various vital functions like blood pressure, central nervous system, liver and kidney function, and metabolism of vitamin D (Singh, A., & Kostova, I. 2024). Thus, it is of utmost requirement to remove heavy metal from the various environmental components**. A** variety of treatment procedures have been used to remediate the heavy metals from soil and water (Rehman *et al.,* 2023).

1.3 Bioaccumulation of heavy metals in fish

Concerns about heavy metal poisoning in aquatic lakes and rivers are significant because it negatively affects the microbes that live there, particularly fish (Jamil Emon *et al.,* 2023). Although metals such as lead are naturally present in the surroundings, their overuse in a variety of sectors for a variety of reasons has drastically changed the balance of nature by causing inappropriate metal discharge into the ground and water bodies (Aljohani *et al.,* 2023). The primary contributors of heavy metals in water bodies are generally thought to be human operations, which include the production of crop foods, field erosion, and the release of domestic and commercial wastes (Patchaiyappan, *et al.,* 2023). Metals such as lead readily accumulate in the various portions of aquatic living animals, including fish, after they invade aquatic environments. They then find their way into the bodies of fish that swallow contaminated seafood (Habib, *et al.,* 2023). Fish health and biological processes are hampered by the accumulation of contaminants in fish (Norani *et al.,* 2023). The type of fish, the concentration of the metals, and the length of exposure all have a substantial impact on the degree of toxicology of the metals (Mehar, *et al.,* 2023).

Fish and other aquatic creatures are susceptible to heavy metal contamination from sediments found in aquatic environments as well as water (Sheikhzadeh, H., & Hamidian, A. H. 2021). Fish with compromised neural systems are less able to interact with their surroundings, as a result of heavy metal-mediated neurotoxicity. Since most of these metals cannot be broken down into harmless states, their unchecked usage and aggregation has

become a major health concern since they can have detrimental impacts on aquatic life as well as human wellness (Agbugui, M. O., & Abe, G. O. 2022). The maximum (ppm) content limits for hazardous pollutants in fish oil capsules are as follows: As -0.1, Pb -0.1, Cd -0.1, and Hg -0.1. The legal allowable limits for the other two items (fish oil enhanced with vitamins and antioxidants) are distinctive and have been established by the manufacturer: Pb is 3, Cd is 1, and Hg is 0.1. Certain heavy metals, including cadmium, lead, and mercury, have no recognized biological functions and are harmful to vital life processes, whereas many other heavy metals are poisonous at only modestly raised free ion concentrations (Arena *et al.,* 2021).

1.4 *Channa punctatus* classification

Predatory fish belonging to the family *Channidae*, it is indigenous to freshwater environments in Asia and has a large natural range that stretches from sections of Russia in the eastern part of Asia to India in the West. Fish are carnivores that eat other fish, frogs, insects, and earthworms as food sources. They also serve as an example of functional foods, which go beyond simple sustenance to offer health benefits. Due to its high content of essential fatty acids and amino acids, there is evidence that it is a good source of therapeutic food (Singh, *et al.,* 2008). Fish also produce glycine, arachidonic acid, and Thromboelastography (TEG), which are medicinal characteristics. Fish mucus, skin, and muscle are used to make fish extracts. Extracts from *Channa punctatus* help with heart health, eye conditions, kidney as well as liver function, and eye problems. As a result of the heavy metals' excessive dispersion into water bodies in the form of colored plastic as well as electronic waste, toxicity is produced by the metals' subsequent biomagnifications in the aquatic fauna and flora (Paul *et al.,* 2014). According to Kato *et al*., (2008) these chemicals caused several disruptions in the organism. For example, chromium IV is very toxic and has the potential to cause cancer. It also caused other ailments, including skin ulcers, sinus carcinoma, and pulmonary sensitization. Cadmium toxicity is the cause of itai-itai disease and renal failure (Nishijo, *et al.,* 2017). Pb intoxication can result in nervous system collapse and mental impairment (Karri, *et al.,* 2016). Because of the large concentrations of these heavy metals that have accumulated, there is significant worry about the possible harm to human health that is directly associated with consuming such contaminated biota (seafood).

As a result, the primary goal of the current study is to ascertain the heavy metal concentration in *Channa punctatus*, which is a popular source of wholesome, nutritious white meat that is consumed by a sizable portion of the global population.

During the industrial revolution, there is a significant concern about maintaining a sustainable and healthy environment. The rapid growth of industries in recent decades has exacerbated this issue, pushing the environment to its limits of tolerance (Kavitha *et al.,* 2010). Among the 70 metals and metalloids found in the environment, 23 are classified as heavy metals/trace metals, and several of them are recognized as potent biological toxins (Goldhirsch *et al.,* 2011). Heavy metals are the primary category of pollutants present in the environment. They can be classified into two groups: necessary metals such as iron (Fe), copper (Cu), and zinc (Zn), and non-essential metals such as lead (Pb), cadmium (Cd), and chromium (Cr). These substances cannot be generated or destroyed and have a direct association with environmental contamination due to their ability to cause toxicity in living organisms (Green, A. J., & Planchart, A., 2018). Heavy metals are generated through both natural and human activities, such as industrial emissions, the presence of contaminants in agrochemicals, traffic, and domestic waste. When these metals are released into the environment without proper treatment or no treatment at all, they pose a significant threat to the survival of living organisms. Heavy metals are chemically stable substances that are resistant to decomposition, have a tendency to build up in sediments, and possess a long half-life in the environment, making them difficult to control. The presence of traits such as bioaccumulation and biomagnifications in living tissues, as well as the inability to be removed through oxidation, precipitation, or bioremediation, make certain elements particularly concerning. Aquatic ecosystems, particularly rivers and oceans, serve as the primary recipients of contaminants and heavy metals. Even minor changes in the environmental quality, including its physicochemical properties, can adversely affect the normal physiology of aquatic organisms, particularly fish, which are highly susceptible to such alterations (Ali, A. S., & US SA, A. R., 2014). They impact aquatic ecosystems either by direct toxicity to live organisms or indirectly by disrupting the food chain (Javed, M., & Usmani, N., 2019). The bioavailability and absorption of heavy metals are influenced by various factors, including the concentration of heavy metals, duration of exposure, interaction with other metals, age and size of the fish, detoxification mechanisms, metabolic processes of

fish, feeding behavior, and physico-chemical parameters of the environment (K AL Taee, *et al.,* 2020).

Fish are the primary consumers in the aquatic food chain and are very suitable for toxicological and toxic genomic research (Chen *et al.,* 2023). Fish possess a high level of sensitivity towards environmental changes, making them excellent bioindicators for monitoring aquatic ecosystems. This is due to their ability to efficiently metabolize, detoxify, and accumulate heavy metals in their bodies (Sirisangarunroj, *et al.,* 2023). Heavy metals can be absorbed by fish through their diet, by taking in water for breathing, or by exchanging ions through a semi-permeable membrane. Once within the fish's body, these metals can accumulate in different tissues (Habib *et al.,* 2024). Fish are regarded as a beneficial source of protein and fatty acids for humans. However, it is important to note that fish have the ability to store heavy metals within their bodies. These metals can then be transferred to the human body, potentially leading to harmful consequences. Similar to larger vertebrates, fish exhibit comparable responses to toxic substances and can be employed to assess the possible mutagenic, carcinogenic, and teratogenic effects of heavy metals on humans (Alam *et al.,* 2023).

Extensive toxicity testing, both acute and chronic, has been conducted on several fish species to assess the impact of heavy metals on aquatic life, particularly fish, in their natural habitat. Fish have a wide range of responses to heavy metal intoxication, which include physiological, biochemical, cellular, and molecular changes within their bodies. Potential biomarkers can be utilized to monitor the presence of dangerous chemicals, such as heavy metals, in the aquatic environment (Elumalai, S., Prabhu, K., Selvan, G. P., & Ramasamy, P. 2023). These metals have the ability to form chemical bonds with biological particles that include nitrogen, sulphur, oxygen, and other elements. This interaction can lead to changes in the structure and function of proteins, enzymes, hormones, and other biomolecules. Ultimately, this can cause harm to various organs in fish (Elumalai, S., Prabhu, K., Selvan, G. P., & Ramasamy, P., 2023). Blood is a crucial biological element that allows for the relatively simple detection of any modifications in hematological parameters (such as RBC, WBC, Hb, Glu), enzyme and hormone levels (such as Alanine transaminase (ALT), Aspartate transaminase (AST), Gamma-glutamyl transferase (GGT) and Cortisol) within the fish's body as a result of exposure to heavy metals. Several studies have documented the

response of blood chemistry and biochemical parameters in various groups of fish raised in different concentrations of heavy metals (de Mendonça *et al.,* 2023). Non-essential heavy metals are often either detoxified or accumulated in the body. Additionally, necessary metals that exceed the acceptable limits also collect in various organs such as the gills, liver, kidney, muscle, gut, skin, and bones. The failure of detoxification processes leads to the accumulation of heavy metals in different organs of fish, resulting in varying degrees of pathological alterations in tissues such as gills, liver, and kidneys.

The literature contains multiple descriptions of the term "heavy metal". It is often used as a substitute for trace metals and includes both essential and nonessential elements that have a high atomic weight and greater density than water. Heavy metal is a word that refers to any substance capable of donating electrons and generating ions with a specified valence. These chemicals have the ability to substitute hydrogen ions in acids, produce compounds when they react with nonmetals, but they are unable to react with other heavy metals. In addition, alkaline oxides are present in heavy metals. In scientific terminology, a metal is defined as a substance that has the capacity to effectively conduct heat and electricity, can be moulded into metal plates and wires, has a metallic colour and lustre, and is usually in a solid state under normal conditions, except for mercury (Prabakaran, 2024). Regardless of its exact categorization, any metal can be categorized as heavy metal if it poses danger to any organism under any circumstance. Heavy metals occur naturally in different quantities in the Earth's crust, soil, air, water, and all living organisms. They have also been extensively dispersed as a result of human activities such as cement production, iron and steel industry. Additionally, they can be spread through natural processes such as wind, soil erosion, and volcanic activity. The exponential rise in agricultural activities, population expansion, urban development, and industrial advancement has resulted in pollution and its related hazards, which are major environmental issues (Azar, H., & Vajargah, M. F., 2023). Without a question, heavy metal contamination is the most perilous type of chemical pollution in water (Azar, H., & Vajargah, M. F., 2023).

Heavy metal toxicity refers to the harmful effects caused by the presence of high levels of heavy metals in living organisms. These metals have the tendency to accumulate, or build up, in the bodies of living creatures. Heavy metals exert a significant impact on the stability of ecosystems and also have detrimental impacts on human health (Shahjahan, C.

2022). While certain heavy metals like zinc, iron, cobalt, and copper are necessary for enzyme activity and other biological activities in small amounts, they become hazardous when they above a specific threshold. In contrast, metals like lead, cadmium, and mercury do not serve any necessary function in living organisms and are dangerous even at very low levels (Taslima, *et al.,* 2022).

1.5 Impact of Heavy Metals on Fish

Certain aquatic creatures have the ability to accumulate and retain heavy metals up to a specific threshold. Although these heavy metals are not inherently hazardous or toxic, they have the potential to be transmitted to people through the food chain and impact human health (Merlini, 1971). Toxicity often occurs when the concentration of heavy metals exceeds certain thresholds. Furthermore, the accumulation of heavy metals in water poses a significant risk to the food chain at many levels, so endangering the safety of the environment, as well as the health of fish and humans (Rehman, *et al.,* 2021). Fish occupy the highest position in the aquatic food chain and have the ability to gather existing metals in different tissues and organs (Sonone, *et al.,* 2020). Aquatic creatures, including fish and shellfish, have the ability to store metals in their bodies at concentrations that are significantly higher than those found in the surrounding water or sediment (Sonone, *et al.,* 2020). The accumulation of metals in fish tissues to toxic levels is influenced by various environmental factors, including the food chain, competition among predators, water chemistry (salinity, pH, water hardness), and fluid dynamics in the water (Sonone, *et al.,* 2020). Moreover, the interaction among metals can potentially have an impact on the accumulation process (Sonone, *et al.,* 2020). Research conducted on fish has shown that heavy metals, even though some are necessary for life, negatively impact living species by disrupting metabolism and causing genetic mutations. The detrimental effects mentioned include a reduction in fitness, disruption of reproduction leading to the development of cancer, and ultimately mortality (Kumare *et al.,* 2023).

Heavy metal effects are an additional factor that causes stress in fish, along with reproduction, hypoxic circumstances, high stocking, and malnutrition (Sonone, *et al.,* 2020). Pollution is a stress factor that negatively impacts growth, development, and reproduction by altering metabolic, physiological, and biochemical activities (Liu, *et al.,* 2020). Observations

have been made of negative effects on the physiological functions and biochemical characteristics of fish living in waters contaminated with metals, both in their blood and tissues. Studies have indicated that fish that were exposed to metals experienced immune system dysfunction, making them more susceptible to contagious diseases and increasing their risk of mortality (Liu, *et al.,* 2020). Although the exact knowledge on the carcinogenic effects of heavy metals is limited, some studies indicate the presence of genotoxic effects (Taslima *et al.,* 2022). Heavy metals can increase the genotoxicity of other chemical agents, either by directly causing toxicity or indirectly by generating toxicity in those chemicals (Bolognesi *et al.,* 1999). Exposure to heavy metals decreases the release of oestrogen and androgen hormones and also leads to pathological alterations in fish (Yilmaz, 2020).

Cadmium is very hazardous, even at low quantities, and has both immediate and long-term consequences on fish and the environment. Prolonged exposure to cadmium has a range of immediate and long-term consequences on aquatic organisms (Kavitha, T., & Sridharan, D., 2010). The effects include an increase in the humoral immune response, as well as changes in the structure and function of the gill, intestine, liver, and kidney. These changes can lead to pathological alterations in the liver, such as congestion, necrosis of pancreatic cells, and fatty changes in the per pancreatic hepatocytes. Additionally, there may be congestion and engorgement of blood vessels. Additionally, it results in disturbances in calcium metabolism, excessive excretion of calcium in the urine (hypercalciuria), and the formation of kidney stones. Fish exhibit varying levels of toxicity, with salmonids being particularly vulnerable to cadmium exposure. Additionally, they may experience sublethal consequences, such as noticeable spinal deformities. Kumar and Sing state that it modifies the antioxidant defence system and the generation of free radicals. Çiftçi *et al.,* (2017) reported a reduction in the hepatosomatic index of Clarias lazera, a species of North African catfish, following exposure to Cd. In the rosy barb fish (*Puntius conchonius*), exposure to high concentrations of Cd resulted in short-term hyperglycemia, while long-term exposure to low concentrations of Cd produced hypoglycemia. Additionally, both conditions led to increased liver glycogen concentrations (Çelik *et al.,* 2008). Witeska and Jezierska (1994) discovered that the red blood cell count and haematocrit levels of common carp (Cyprinus carpio) exposed to Cd rose. In a study conducted by Johansson-Sjobeck and Larsson in (1978), it was demonstrated that the red blood cell count, haematocrit, and haemoglobin

levels of European flounder (*Pleuronectes flesus*) had a considerable drop following exposure to Cd. In Mozambique, the presence of Cd in tilapia (*Oreochromis mossambicus*) resulted in a reduction in haemoglobin level s and red blood cell count, as reported by Ruparella *et al.,* (1990) and Çelik (2006).

Tort *et al.,* (1988) discovered that exposure to Cd led to a decrease in the concentration of white blood cells (leucocytes) in the lesser spotted dogfish (Scyliorhinus canicula). Kavitha, T., & Sridharan, D. (2010) obtained same findings in Mozambique tilapia. Nevertheless, Tort and Hernandes-Pascual (1990) noted a reduction in the white blood cell count of Mozambique tilapia that was exposed to Cd (Çelik, 2006). Furthermore, Cd has an impact on the glucose levels of fish. Research has demonstrated that exposure to Cd led to an elevation in glucose levels in rainbow trout (*Oncorhynchus mykiss*) (Haux and Larsson, 1984), whereas it caused a reduction in glucose levels in common carp (Cyprinus carpio) (Kavitha, T., & Sridharan, D. (2010). Çelik (2006) demonstrated that exposure to Cd in common carp resulted in an increase in glucose levels on the 1st and 3rd days, but there was no impact on glucose levels on the 15th and 30th days following exposure. The kidney is the primary organ affected by cadmium toxicity and chronic exposure in nearly all animal species. This exposure leads to varied levels of renal impairment, as documented by several studies (Kavitha, T., & Sridharan, D. (2010). Copper negatively affects the resistance of fish to diseases by interfering with their migration, altering their swimming behavior, causing oxidative damage, impairing their respiration, and disrupting the structure and function of important organs such as the gills, kidney, liver, and stem cells (Kavitha, T., & Sridharan, D. (2010).

Exposure to copper resulted in various fish species exhibiting behavioral alterations, including reduced swimming ability and food intake, as well as increased operculum motions (Green, A. J., & Planchart, A., (2018). Arslan *et al*., (2020) discovered that these alterations returned to their original state with extended periods of exposure. The presence of copper in stinging catfish, rainbow trout, and North African catfish resulted in a decrease in muscle and liver glycogen levels, whereas serum glucose levels increased (Green, A. J., & Planchart, A. (2018). Arslan *et al.,* proposed that these modifications may have occurred as a result of fish adapting to hypoxic circumstances caused by the presence of copper. Javed, M., & Usmani, N., (2019) shown that copper has a negative impact on many fish species, resulting in a

reduction in the overall protein content in the muscle and liver, as well as an increase in the concentration of free amino acids and the activity of gluconeogenic enzymes (Javed, M., & Usmani, N. (2019). Çiftçi *et al.,* (2017) conducted a study on the impact of copper (Cu) on nile tilapia (*Oreochromis niloticus*) in relation to the hepatosomatic index (HSI), gonadosomatic index (GSI), and condition factor (CF). The results revealed that Cu led to an increase in HSI and a decrease in CF. Çiftçi *et al.,* (2017) discovered that whereas Cu initially caused an increase in GSI, prolonged exposure resulted in a subsequent drop in GSI. Fish depend on their olfactory sense to undertake migration, evade predators, and locate sustenance. Copper impacts the olfaction of fish, leading to changes in hunger, navigation, and perception of the environment. Additionally, it diminishes the generation of sperm and eggs, lowers survival rates, and raises the occurrences of abnormalities (K AL Taee, *et al.,* 2020).

Heavy metals, a major concern in aquatic fauna studies, are causing significant damage to surface and groundwater quality due to extensive garbage dumping and chemical seepage. These pollutants accumulate and increase in concentration, affecting physiological, cellular, and biochemical processes (Javed, *et al.,* 2019; Bashir, *et al.,* 2020; Choudhary, *et al.,* 2012 and Ali, *et al.,* 2019). The United States Environmental Protection Agency (USEPA) and Environmental Protection Agency (EPA) express worry with the presence of lead in drinking water due to its high level of toxicity. The sediments of Gomati and Kali River have elevated levels of lead, posing a threat to aquatic plants, animals, and other organisms due to the combined impact of heavy metals, bacteria, medicines, coliforms, microplastics, and pesticides (USEPA, 2012; Mitchell, *et al.,* 2018; Neha, *et al.,* 2017; Malik, *et al.,* 2014; Manikandan, *et al.,* 2016 and Nigar, *et al.,* 2017). Fish, being aquatic species, serve as indicators of heavy metal pollution, specifically lead (Pb), which is a hazardous and persistent pollutant in bodies of water. Lead (Pb) is second on the top 20 list of the Agency for Toxic Substances and Disease Registry (ATSDR) and presents a substantial environmental hazard through its presence in automobile exhaust pipes, fuel, and paint (Łuczyńska, *et al.,* 2008; Ahmed, *et al.,* 2019; Baby, *et al.,* 2010; Sall, et *al.,* 202; ATSDR, T. (2000) and Jaishankar, *et al.,* 2014). Industrial activities like plumbing, ammunition, battery manufacturing, and toy production contribute to lead pollution. Lead, a divalent cation in freshwater, is a hazardous environmental toxin due to its high bioavailability and

toxicity. This study investigates the acute toxicological effects of lead nitrate exposure on two fish species, C. punctatus and H. fossilis, found in freshwater environments (Nigar, *et al.,* 2021; Verma, *et al.,* 2013; Srivastav, *et al.,* 2013; Senthamilselvan, *et al.,* 2015 and Rani, *et al.,* 2011).

1.6 Gomti River: Flow Along a cross

The Gomti, Gumti or Gomati River is a tributary of the Ganges. According to the Bhagavata Purana, one of the fundamental sacred texts of Hinduism, the Gomti River is one of India's five transcendental rivers **(**Jain, 2020). It is a monsoon- and groundwater-fed river that originates from Madho Tanda in Pilibhit, India, from the Gomat Taal (formerly known as Fulhaarjheel). It travels 960 km (600 mi) through Uttar Pradesh until coming along with the Ganges in Saidpur (Ghazipur district), Kaithi, 27 km (17 mi) from Varanasi. The Gomti approaches Lucknow after travelling 190 kilometers (120 miles), meandering through the city for around 12 kilometers (7 miles), and provides its water to sustain several activities (Abey singha *et al.,* 2016). Due to the discharge of sewage and industrial effluent, pollution is posing an onerous barrier in Gomti. 25 city drains in the Lucknow region empty raw sewage into the river. The Gomti barrage changes the river's downstream end into a lake (Sharma, M. 2005). According to earlier assessments of water quality conducted by the Uttar Pradesh department of irrigation in 2016 and 2019, respectively, Gomti water has a significant level of pollutants (Krishan, *et al.,* 2022). While enduring the pressure of expanding human activities, surface water sources have undergone numerous quantitative and qualitative alterations. There has been reported a constant increase in the levels of heavy metal (HM) pollution and deterioration of river water quality because of the discharge of untreated or only partially treated industrial and domestic effluents into rivers **(**Kumar, *et al.,* 2024). HMs is potentially hazardous to humans and other biotic components since they are toxic and non-biodegradable in nature (Kakade, *et al.,* 2023).

The research exhibited that there is a clear correlation between the population increase, rapid urbanization, and a lack of effective wastewater treatment systems, which results in heavy metal pollution in rivers (Kakade, *et al.,* 2023). The disposal of municipal and urban waste into river catchments in developing countries like India has arisen as a significant concern in maintaining river water quality. Humans and animals both face major

health risks from the bioaccumulation and biomagnifications of HMs in the food chain **(**Ali *et al.,* 2021). The serious health risks linked to lead (Pb) intake through the food chain have also been emphasized. Therefore, regular monitoring and efficient management techniques for water quality of surface sources are crucial for survival of living organisms. Constant monitoring of metal deposition in river water and sediments allows for the determination of the acceptability of river water for operations **(**World Health Organization, 2016). Through their concentration in water and sediment, HMs is found in aquatic environments and their source and movement are identified. Therefore, it is crucial to monitor the quality of river water along its path, particularly if it is used as a source of drinking water and is subject to anthropogenic interference. To ensure the viability of river in ecosystems, it is necessary to design a long-term adaptation strategy that considers environmental flows and ecosystem services **(**Arthington *et al.,* 2010).

1.7 Hypothesis

At various trophic cascades in the food chain, lead is a continuous environmental adulterant and requires specific attention because of its high toxicity and long-term persistence in the environment. It is generally known that long term exposure of lead in humans can result in anemia, impaired fertility, renal failure, and neurological damage. Increased lead deposition in aquatic environments may result in increased lead permeability within fish bodies.

1.8 Objectives of the Study

In this regard, the present study is proposed to pursue research with the following objectives in mind:

1. Analysis of non-essential heavy metals in water and sediment of selected rivers,
2. To estimate the bioaccumulation concentration factor of selected heavy metals in edible fish,
3. To analyze the toxic effects of heavy metals on hematological, histopathological, and biochemical parameters of fish and the use of fish oil in herbal products and,
4. Analysis of molecular pathway involved in selected heavy metals toxicity.

Chapter-2

REVIEW OF LITERATURE

Metals play a crucial role in biological systems because a living cell cannot perform well without metal ions. The modern periodic table's d-block elements comprise heavy metals, commonly referred to as transition elements. These elements are five times more dense than water and have high atomic densities (>5 kg cm^{-3}) (Manara, 2012). The densities of different heavy metals are given in Table 2.1.

Table 2.1 The densities of different heavy metals, (Source: Tchounwou, *et al.,* 2012)

Lead (Pb)	11.3
Zinc (Zn)	9.16
Cadmium (Cd)	8.7
Arsenic (As)	5.8
Aluminums (Al)	2.7

Many heavy metals, including Zn, Cu, Ni, Fe, and Mn, are necessary metal ions for living organisms in trace amounts and are crucial for human health. However, metals can result in major health issues if they are consumed and accumulate at a high concentration (Alloway, 2012). Due to their extensive use, distribution, and notably their toxicity to humans and other living things, heavy metal poisoning is a prevalent environmental issue that poses a serious health risk and necessitates high cleaning expenses (Lenart-Boroń, & Boroń, 2014). There has been a direct impact on the environment due to the rapid industrialization, development, and urbanization as well as the explosion in human population growth, which has ultimately resulted in the degradation and contamination of the environment and poses a serious health risk to all life forms on earth (Ukaogo, *et al.,* 2020). Through a variety of human activities, such as the release of garbage and other pollutants into the environment either directly or indirectly, land, air, and water have become contaminated with heavy metals (Sankhla, *et al.,* 2016). The accumulation of untreated harmful substances from the environment in the food chain causes serious health issues. There has been a notable

surge in studies due to heightened consciousness regarding the detrimental consequences of environmental contamination, especially that caused by metals.

This research was initially done to learn more about the negative effects of heavy metal ions on the microbiota in contaminated soil and water, and then to determine how to remove these ions from environmental components (Salama, *et al.,* 2019). Heavy metals are significant pollutants that come from both direct and indirect sources, including sludge, industrial effluents, sewage, household waste, municipal trash, agricultural waste, and mine tailings **(**Wang, & Wang, 2015). Even though the presence of heavy metals has been found in home and industrial waste, solutions are still required to address the issue of heavy metal contamination, particularly in water. To remove, recover, and stabilize heavy metals found in waterways and effluents, researchers and industrialists have developed a keen interest in the interaction between metals and microbes in recent years (Adkins, 2019).

1.5.1Major sources of lead contamination

Biologically Lead (Pb) is a non-essential element. This metal, which is a major contaminant and a hazardous waste, can be found in soil, water, and the air. It is extremely harmful to humans, animals, plants, and microorganisms (Chatterjee *et al.,* 2012). Petroleum, the electronics industry, batteries, paint, stained glass, and the manufacture of biocides are among the main sources of lead (Kushwaha, *et al.,* 2017). One of the main causes of lead pollution in cities around the world is the use of lead petrol in automobiles (Cheng & Hu 2010). Road traffic is thought to contribute more than a thousand tons of lead per year to the environment because of lead content present in petrol (Mielke *et al.,* 2011). These air emissions are primarily absorbed by water, plants, and food (Fritioff, & Greger, 2006). According to a study conducted on smoked fish exposed to motorways, there is a correlation observed between the amount of traffic on the roads and the Pb levels in the fish. This poses a potential risk to the well-being, safety, and security of these food products (Adekunle, & Akinyemi, 2004). The most potential sources of lead contamination are:

1.5.1.1 Natural sources

Furthermore, due to its extremely concentrated ores, lead is naturally found in very small levels in all environments. Lead finds its way into the environment, water, and plants

through both wet and dry atmospheric deposition. Submicron-sized lead particles, which are a significant portion of emissions, can travel over great distances. Larger lead particles gravitate toward the source and settle there more quickly. Thus, new depositions primarily atmospheric contribute to rising lead concentrations. The main source of lead in surface water is the atmospheric deposition of lead particles (Ali *et al.,* 2020).

1.5.1.2 Anthropogenic sources

Human-induced causes include burning fossil fuels, antifouling finishes, municipal and agricultural drainage, and household and commercial wastewater can cause amounts of metals in the water supply to exceed baseline levels (Fuge, 2013). As a result of mining and smelting operations, the burning of leaded fuel, the spreading of sewage sludge and the dumping of batteries and other Pb-containing goods, high quantities of Pb are abnormally discovered in soil and water (Lin *et al.,* 1998). Various sources of lead contamination in domestic sewage are mentioned in Table 2.2 and Figure 2.1.

Table 2.2 Heavy metals released from domestic waste water (Masindi, & Muedi, 2018; Margot, *et al.*, 2015).

Automotive Products	Al, As, Be, Bi, Cd, Co, Cr, Fe, Pb, Mo, Mn, Hg, Ni, Se, Sn, Ti.
Caulking Compound	Al, Be, Cr, Fe, Pb,
Cleaners	Pb, Al, Fe,
Cosmetics	Al, Be, Bi, Cd, Co, Cu, Fe, Pb, Mn, Hg, Ni, Se, Ag, Sn, Ti, V, Zn.
Disinfectants	Hg,
Water treatment	Al, Co, Fe. Mn, Zn.
Preservatives	Fe, Pb, Zn.
Powders	Al, Fe, Ag, Ti, Zn
Paints	Al, As, Be, Cr, Co, Fe, Pb, Mn, Hg, Ti, Zn
Photography	Al, Cd, Cr, Co, Fe, Pb, Hg, Ag, Sr,
Paper	Al, Sb, As, Be, Bi, Cd, Cr
Leather	Pb, Ag, Sn, Ti.

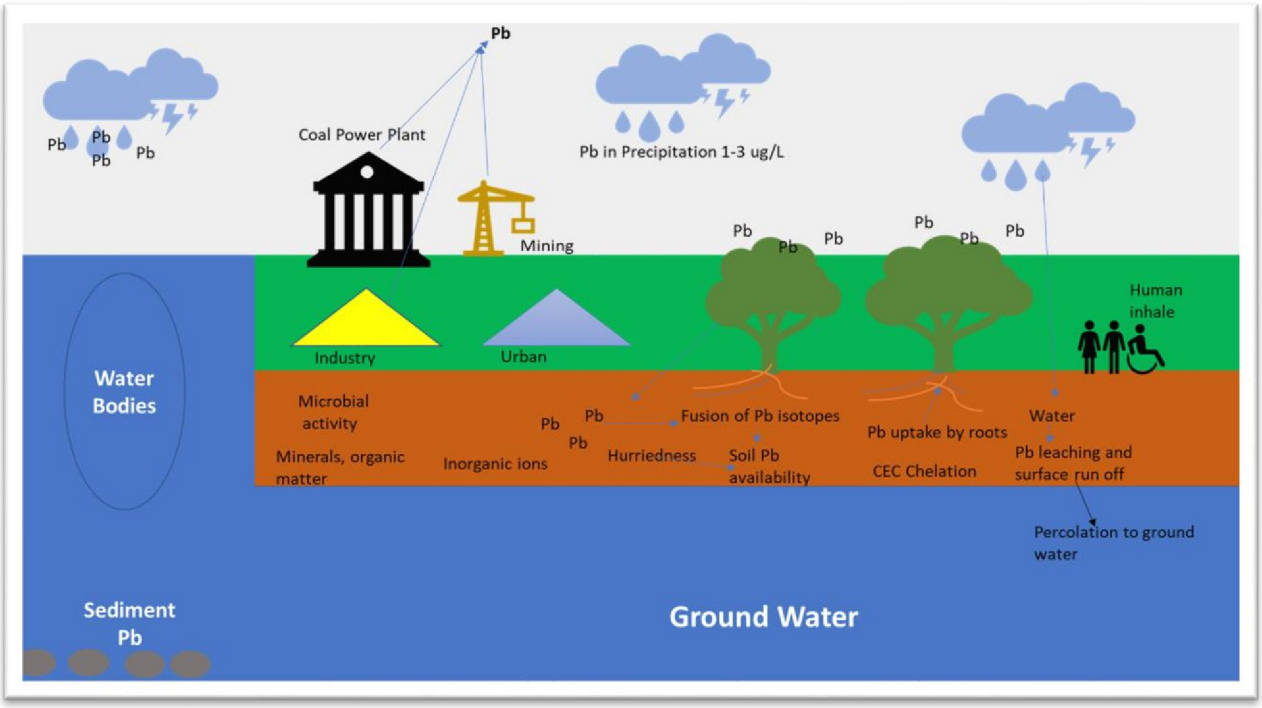

Figure 2.1: Lead Cycle in Environment (Margot, *et al.*, 2015).

1.5.2.3 Effect of Lead on Renal System

Numerous investigations have discovered a link between low environmental lead levels of exposure and a quicker onset of chronic kidney failure. Even at significantly lower levels than the upper bounds of the usual ranges in people of all ages, the levels of blood lead were found to be boosted which indicated a quicker onset of chronic renal failure (Wang *et al.,* 2022). Lee *et al.,* 2022) studied the effects of occupational lead hazards on patients. In individuals who underwent a biopsy, they noticed tubular dysfunctions. According to this research, lead nephropathy may be a serious occupational risk. Lead poisoning patients were observed to excrete the waste product urate, suggesting gout, in which the body accumulates urate **(**Rubin, & Strayer, 2008).

1.5.2.4 Effect of Lead on Pathophysiology

Lead levels may also rise as a result of direct or indirect contact with lead or lead-based compounds through the mouth, nose, eyes, or skin crevices. Amounts of inhaled lead dust that are deposited in individuals' lungs range from 35 to 40 percent, with 95 percent entering the bloodstream. While lead is absorbed at a high rate approximately 94% in bones and teeth in children, it is only absorbed at a rate of 70% in adults. This permits the soft tissues to absorb more lead, which can have major health effects **(**Barbosa *et al.,* 2005). In humans, the half-life of lead is predicted to be approximately 40 days. This is more likely to happen to pregnant women and young children whose bones are still forming. Lead can be repeatedly reintroduced into circulation due to the growth and remodeling bones of youngsters **(**Barbosa *et al.,* 2005).

1.5.2.5 Effect of Lead on Children

Pregnant mothers who have high levels of lead in their bodies run the risk of giving birth to premature babies or children who are underweight. Fetal lead levels significantly lower than 25 milligrams per deciliter of blood may be hazardous. At the same time, it was found that the neonate's levels of lead in his blood were higher than the mother's (Bellinger, 2005). It is believed that emaciated women who were heavily exposed to lead before becoming pregnant are more vulnerable. Children's bodies are still developing and growing, which has been linked to an increased risk of ingesting lead. Furthermore, children consume

lead more rapidly than adults do. Due to their undeveloped behaviors, children are more prone to take in and swallow lead-contaminated dust.

Bioaccumulation

According to Eroglu *et al.,* (2015), the main causes of metal toxicity in fish are bioaccumulation in addition to elimination, metabolism, and detoxifying processes brought on by metal uptake. Metals are ingested by fish through their gills and food, which are then excreted into the stomach by the liver following detoxification. The liver's role in binding metal to corticosteroids in the bile is a critical part of fish susceptibility to lead purification systems. After that, the bile-metal (Pb) compounds are either expelled with feces or digested by the digestive tract wall. The cardiovascular system allows metal that reaches the body via the gills as well as the liver to exit the body. Certain metals are eliminated from the body through the urinary tract and gills, while other metals build up and are kept in tissues or harmful to certain tissues. Because of its ability to quickly bind oxygen and sulfur atoms in amino acids and create stable complexes, lead (Pb) is one of the most dangerous metals that accumulate over time. Previous research has shown a significant buildup of Pb in a variety of fish tissues. Dietary lead exposure caused a notable build-up in the kidneys, liver, spleen, gut, and gills of young rockfish, *Sebastes schlegelii*. Hwang and colleagues also documented a noteworthy build-up in the gut, kidney, such as liver, and gills of *Platichthys stellatus*, a starry flounder that was subjected to dietary lead exposure.

Iono-regulatory disruption brought on by prolonged Pb exposure affects the homeostasis of $Ca^{2+,}$ K^{+}, and Na^{+}. Ca^{2+} and Pb^{2+} have an antagonistic connection with one another among the ions. Because Ca2+ helps fish reduce body Pb deposition by adhering to suspended ambient Pb, it affects Pb toxicity in fish exposed to both acute and chronic Pb exposure. Furthermore, dietary Pb accumulation in tissues is prevented by Ca^{2+}. The gills and intestine's Ca^{2+} regulatory system, which control the absorption of Pb in tissues, are what produces this protective effect. According to Ahmad et al. (2012), Ca^{2+} is implicated in the elimination of metals like Zn, Pb, Cu, and Fe. The order of all of these metals' elimination was Pb^{2+}> Ca^{2+}> Zn^{2+}> Cu^{2+}> Fe^{3+}> Cr^{3+}.

Understanding the pathways of exposure is crucial to understanding the mechanisms by which metal exposure leads to buildup, as variations in dietary or aquatic metal intake can

significantly affect accumulating trends. Since the gill tissue is subjected to metallic ions in water bodies during breathing and oxygen regulation, rather large rates of deposition may be seen in the event of environmental exposure. However, in the event of food exposure, tissue in the intestines exhibits substantial rates of aggregation.

Immune response

Fish immune system responses are altered when exposed to lead as an environment immuno-toxicant. Additionally, Pb alters immune system function in animals, leading to physiological nature, biochemical processes, and neurological problems. Dunier (1996) proposed that fish exposed to Pb experience drop-in phagocyte activity, reduced antibody generation, and lowered blood vessel activity in the spleen. According to Adeyemo *et al.,* (2010), lead exposure in fish can stimulate the body's defenses and produce tissue damage, which can increase the number of lymphocytes. Nevertheless, persistent exposure reduces the number of lymphocytes and white blood cells because of immunological system impairments. Fish exposed to lead saw a significant drop in the number of white blood cells and lymphocytes; this was due to the stress response's induction of cortisol release, which shortened lymphocyte life spans or encouraged apoptosis. Zelikoff (1993) found that Pb injection dramatically reduced antibody titers in brown trout in dose-dependent ways.

By regulating the production of cytokines, lead can influence an immunological response. Interleukins (ILs) and tumor necrosis factor (TNFs) are examples of cytokines, which are proteins that control messages between different cells to trigger a protective response and are crucial in controlling the immune system. Amongst these cytokines, tumor necrosis factor-α (TNF-α) is engaged in inflammation, a process called the immunological response, while interleukin ten (IL-10) is implicated in the cellular aggressive immune system response. By stimulating the activity of mitogen-activated protein kinases (MAPKs), lead (Pb) modifies the immunological response. MAPKs are crucial for conveying several signals, including those related to apoptotic and stress reactions, to the nucleus. Three individual components make up MAPKs: c-Jun amino-terminal kinases, and exogenous signal-regulated kinases. JNKs control cell survival and apoptotic; p38 plays a role in both inflammatory and apoptotic; and ERK promotes proliferation and development. Furthermore, Pb also increases fish Hsp70 transcription. Important roles played by Hsp70 include transport

within cells, chaperoning, and the generation of tumor-specific antigens by stimulating T and NK cell immunity. Moreover, Hsp70 stimulates the release of cytokines including TNFs as well as ILs.

Numerous investigations have demonstrated that Pb adversely affects immunological responses in fish, including lymphocytes, leukocyte inflammatory processes, and apoptosis. In addition to suppressing the function of many biomolecules, lead poisoning also affects the immune system by interfering with intracellular signal transduction. As a result, modifications in the fish immune systems have been utilized as a key marker for identifying the harmful consequences of lead exposure.

Bioaccumulation evaluation is a crucial indicator to track the geochemistry cycle of contaminants in the marine environment. The harmful impacts and combustion of the metals differ depending on their distinct forms and types of metal. Chromium (Cr) is often found in six different oxidation states (+1 to +6), with hexavalent Cr being particularly harmful to fish. Fish in lakes and rivers polluted with heavy metals present a significant risk, as they accumulate metal in several vital tissues such as gills, liver, kidney, skin, and muscle. Fish need additional energy to adapt to this stressful state, which they obtain from stored resources such as proteins, lipids, and carbs. Certain metals (such as Arsenic, Cadmium, Chromium, Copper, Iron, Mercury, Nickel, Lead, and Zinc) possess redox potential and can generate reactive oxygen species (ROS). These ROS are crucial for maintaining specific physiological processes in fish. ROS serves as a marker of oxidative stress, which hampers cellular activity by breaking down proteins, lipids, and DNA. Heavy metals accumulate in various aquatic creatures in the food chain and pose significant health risks to humans when consuming polluted fish (Sharma, V., & Mehdi, M. M. (2023).

Mercury is a highly hazardous heavy metal that contaminates water bodies through both natural and human activities (Burke, *et al.,* 2022). Studies have indicated that inorganic arsenic is more hazardous than its organic counterparts the accumulation of heavy metals in various organs of fish occurs at varying rates (Chen, *et al.,* 2023). The liver collected the maximum quantity of as (10.04 ± 2.99 μg/g), while the smallest amount (3.74 ± 3.38 μg/g) was seen in the skeletal muscle of *Oreochromis niloticus* after 20 days after exposure (Sharma, 2023). Multiple studies have documented that exposure to As has resulted in a range of detrimental effects on fish, including decreased growth and productivity, alterations

in blood and metabolic parameters, disruptions in hormone function, abnormal tissue changes, delays in embryonic and larval development, and the occurrence of numerous diseases (Habib, 2024). Furthermore, the haematology and immunology of multiple fish species were considerably impacted by toxicity, as evidenced by studies (Burke, *et al.,* 2022). An elevated concentration of as led to increased mucus secretion, atypical swimming behavior, and impaired equilibrium in *Anabas testudineus* and Danio rerio (Habib, 2024). Stimulation resulted in the creation of apoptosis, tiny nuclei, and various irregularities in the cells and nuclei of fish erythrocytes. It has been observed that as caused multiple harmful effects on the cells and genes of medaka fish, scientifically known as *Oryzias latipes*. Furthermore, pollution hindered the reproduction processes of fish by impeding the procedure of gametogenesis, leading to a detrimental impact on both the quantity and quality of sperm and eggs, as well as on the success of fertilization and hatching.

Cadmium is very poisonous and carcinogenic to human beings and various creatures, especially fish. a report from the United States Agency for Toxic Substances and Diseases Registry, this metal is classified as the eighth most dangerous substance (Elumalai, 2023). Multiple investigations have indicated that the water around us is heavily polluted with Cd (Elumalai, 2023). A variety of aquatic organisms have experienced absorption and the bioaccumulation of this harmful metal. The toxicity of Cd has led to the impairment of vital organs in fish, including the liver, kidney, and gills, which disrupts their physiological functions and impedes their growth (Zheng, *et al.,* 2023). In addition, Cd disrupts iron metabolism and causes anemia, leading to changes in hematological indices (Zheng, *et al.,* 2023). Cadmium (Cd) leads to the suppression of antioxidant enzymes, resulting in the initiation of peroxidation of lipids in animals. In addition, the toxicity of Cd has a detrimental impact on the reproduction capacity of fish. It causes a reduction in the size of sperm lobules, the development of fibrosis in the testes, and a decrease in the movement of sperm and survival (de Mendonça *et al.,* 2023). Chromium (Cr) is a widely distributed metal that contributes to environmental degradation, originating from several industrial sectors. Multiple investigations have documented the bioaccumulation of chromium (Cr) in various organs of different fish species, including *Cyprinus carpio*, and *Cirrhinus mrigala*. The presence of chromium toxicity disrupts the normal physiological processes of fish and leads to a range of allergic reactions and failures in different organ systems (Prabakaran, *et al.,*

2024). Moreover, chromium toxicity has a profound impact on protein, lipids, and glycogen stores in the muscles, liver, along with gills of *Labeo rohita* and *C. mrigala*. It induces hepatic stress in C. *auratus*, disrupts the proper functioning of vital organs such as kidneys and liver in *Ctenopharyngodon idella*, and leads to abnormal functioning of the endocrine system in various species of freshwater fish. Research has shown that Cr can modify the blood profile of *Pangasianodon hypophthalmus*, leading to anomalies in the cells and nuclei. Multiple studies have documented that elevated amounts of chromium in fish diets have a considerable negative impact on the growth and efficiency of food utilization in various fish species. Furthermore, prolonged exposure to chromium (Cr) led to complications in fish reproductive, as evidenced by reduced spawning success, testicular deformities, decreased sperm motility, and impaired ocyte development.

Copper (Cu) is a substantial pollutant in aquatic systems, causing stressful conditions for aquatic animals and greatly impeding the growth and metabolism of fish (Shahjahan *et al.,* 2022). The buildup of copper (Cu) in several tissues of different kinds of fish. Multiple investigations have shown that the liver is the primary organ where a substantial amount of copper accumulates, in contrast to other organs. The presence of excessive amounts of copper in the fish diet resulted in a decrease in the fish's appetite, leading to a negative impact on the fish's ability to efficiently use the feed and grow. Furthermore, the toxicity of copper not only caused abnormalities in the reproductive system but also significantly decreased the gonadosomatic index (GSI), fecundity, fertilization, and hatching rate of many fish species (Yilmaz, *et al.,* 2020).

Manganese (Mn) is ubiquitously present in various contexts. Manganese (Mn) has been seen disintegrating into bodies of water as a result of several human activities. Manganese toxicity in fish can vary due to several factors, such as the type of fish, age, and the water's condition. Research has shown that the toxicity of Mn decreases when the water hardness increases. The presence of Mn in the liver, the gills and muscle of Argyrosomus japonicas resulted in the buildup of this substance. This accumulation disrupted the breakdown of carbohydrates and caused changes in the ionic composition of the blood plasma. Manganese (Mn) has a significant impact on the physiological processes of fish and can occasionally result in severe and lethal consequences. Exposure to Mn leads to oxidative stress in C. auratus. Manganese (Mn) leads to numerous neurogenetic diseases by promoting

the production of radicals that are free and deactivating various enzymes linked to antioxidant capabilities. In addition, Mn has hepatotoxic effects and triggers cell death in grouper Łuczyńska, *et al.,* 2022.

Ni is widely utilized in various industrial processes and is recognized as a predominant pollutant in aquatic ecosystems. In aquatic environments, Ni interacts with other chemical components to create soluble salts that can absorb onto other molecules, resulting in various synergistic as well as antagonistic effects. The degree of Ni toxicity is contingent upon several parameters, including the concentration of Ni, the quality of water, and the physiological condition of organisms. Multiple research projects have shown that fish tend to store Nickel (Ni) in various organs, particularly in the gills. This accumulation of Ni in the gills has been found to cause complications in breathing processes, as reported in studies. Furthermore, it was discovered that Ni tends to build up in the fish's intestine and interfere with its normal functioning (Ture *et al.,* 2021). Ni disrupts the regular physiological processes and leads to the mortality of multiple species of freshwater fish. The presence of Ni pollution causes several histological abnormalities in the gills of *Orechromis niloticus*, such as hypertrophy, hypertrophy, which and fusion of gill lamellae that. In addition, the toxicity of Ni impairs the control of ions and causes oxygen consumption in fish. Two investigations found no statistically significant effects on fish growth nevertheless, they did find significant influences on the emergence of *pulmonate snails* and zebrafish (Ture, M., Kilic, M. B., & Altinok, I. (2021).

Lead (Pb) is a very toxic metal that accumulates in aquatic species through both water and food sources. Lead (Pb) is collected in several organs of fish, such as the liver, kidney, gills, spleen, and digestive system. Lead (Pb) has a considerable impact on the blood parameters of fish, as evidenced by multiple studies. In addition, lead (Pb) toxicity leads to a substantial change in enzyme activity in the blood plasma and liver of fish, resulting in various diseases in the cell membrane and damage to liver cells. Lead (Pb) has a detrimental impact on the growth and feeding efficiency of fish, resulting in decreased weight gain, specific growth rate, increased feed intake. In addition, Pb exposure leads to detrimental effects on reproductive functions, including the production of low-quality sperm as well as ovum, decreased rates of fertilization and hatching, and lower survival of embryos and larvae (Shahjahan, *et al.,* 2022).

Zinc (Zn) is a crucial micronutrient that has a major impact on the growth as well as reproductive of fish. Nevertheless, an excessive amount of Zn can have harmful consequences on fish. The presence of Zn pollution in aquatic habitats has been extensively documented the liver and kidney tissues are the primary locations for the buildup of zinc. Zinc poisoning has detrimental effects on various aspects of fish physiology, including growth reproduction, equilibrium, feed intake, and bone formation. Zinc poisoning leads to the excretion of ammonia, which in turn causes a decline in water quality and creates stressful conditions for fish. Furthermore, Zn poisoning adversely affects the fish liver by elevating the levels of ALT and AST enzymes. In addition, elevated levels of zinc have a substantial impact on the reduction of fish body protein and lipids. This might potentially lead to the oxidation of amino acids and lipids, and possibly a decrease in protein intake (Sadeghi, *et al.,* 2023).

Chapter-3

MATERIALS AND METHODS

Model organism

The toxicity investigations utilized *Channa punctatus* fish, which were 8-10 cm in length and weighed 28 ± 0.6 g. These specimens were obtained from a fish market located in Lucknow, India. The fish were acclimated to the controlled settings of the laboratory for one week. The fish were nourished with conventional powdered feed and underwent a period of fasting lasting 24 hours before the study. The water quality parameters, including temperature, dissolved oxygen, and pH, were maintained according to the recommendations of USEPA, 1986. The metal toxicant utilized was lead acetate of analytical grade (Khan, 2014).

Water sample collection

Sample Collection

Sampling Sites

River Gomati flows centrally through Lucknow city from west to east and spans about 5 km in the city. A total of five sampling sites were selected for regular sampling of water. These are the Vaikunth Dham (site I), Riverfront (site II), Hanuman Setu – (site III), Shaheed Smarak – (site IV) and Dali Ganj- (site V). (Figure 1.1, Table 1.1),

Site I: Vaikunth Dham - This site is situated the upstream before riverfront. The site being unaffected by municipal sewage exhibits pristine river water quality and cremation. The water current is feeble during summer whereas it is much faster during the monsoon.

Site II: Riverfront - This site is located on the southern side of the river, roughly 2 km east and downstream of Vaikunth Dham. The river Gomati is contaminated at this location by two significant municipal drains on the southern bank and one significant drain as well as two smaller drains on the northern bank. At this location, the municipal drains transport sewage and effluents at a rate of 25 MLD (Million Litre Per Day).

Hanuman Setu -This site is 0.5 km east of the first site on the northern bank of the Gomati River, upstream of Vaikunth Dham (Plate 2). A large (Plate 8) and a small drain deliver a murky quantity of sewage to the river. Due to the declining water level of the river during the winter and summer, there is some stagnation in the river's peripheral parts. The municipal drain at this site discharges about 32 MLD of sewage.

Shaheed Smarak - This site is situated downstream of the third site at about 0.5 km on the northern bank of Gomti (Plate 3 & 9). This site receives a large amount of agricultural and municipal run-off from two drains on the north bank and municipal waste from two large drains on the southern bank (Plate 10). It achieves nearly stagnant conditions during the peak summer season due to decreased water levels. The municipal drain at this site discharges about 74 MLD of sewage.

Site V: Dali Ganj- This is the site, situated about 1 km west and upstream from the third site. It is located on the southern bank of the river. This is a site of maximum sewage discharge (Plate 3 & 11). Two large drains on the north bank (Plate 13) and three big drains on the south bank (Plate 12) discharge large amounts of agricultural and municipal wastes which has the largest impact on the river's water quality. During peak summer stagnation is observed. Maximum amount of sewage *i.e.,* about 86 MLD is discharged at this site.

Table 3.1 Location of sampling sites

S.No.	Site Name	Latitude	Longitude
1.	Vaikunth Dham	26.8579° N	80.9671° E
2.	Riverfront	26.8546° N,	80.9706° E
3.	Hanuman Setu	26.8606° N,	80.9374° E
4.	Shaheed Smarak	26.8633°N	80.9284° E
5	Dali Ganj	26.8728°N	80.9276° E

Figure 3.1: Sampling Area Map *(Source: google map)*

(a) Baikunth Dham where the arrow represents the source of heavy metals, due to electric crematorium effluents and Kukrail Nala effluents heavy mental accumulates in Gomati river.

(b) Riverfront where municipal waste drainage releases heavy metals in Gomti River.

(c) Hanuman satu where an arrow representing temple effluents are a source of heavy metals in the Gomti river.

(d) Daliganj satu where fruit market and temple effluents are a big source of heavy metals.

(e) Shaheed Smark where gomeshwar shiv mandir effluents are a source of heavy metals in the Gomti River.

Sampling Procedure

Water samples from the Gomti River were collected in June and July in five replicates from each of the sampling sites in clean 2.0 L pre-sterilized plastic bottles. River water samples were taken from 5m downstream about 2m toward the mid-stream from the point where the drain mixes with river water. The temperature of water samples was reported at the sampling sites instantly with the help of a Celsius thermometer. Transparency of river water was also measured at the sampling sites with the help of a Secchi disc. To evaluate the dissolved oxygen, samples were fixed immediately at the sampling sites with manganous sulfate and alkali iodide azide. For bacteriological analysis, triplicate samples of river water were aggregated in sterile glass bottles and instantly shifted to the laboratory in an ice bucket at 4^0 C. The cold chain was sustained throughout transportation to the laboratory of Integral University, Lucknow, India. Before and during the studies, collected samples were kept at 4 degrees Celsius.

Physico-Chemical Analysis of collected water samples:

A physicochemical analysis was executed on water samples. Eight physicochemical factors, including turbidity, pH, temperature, hardness, total dissolved solids (TDS), dissolved oxygen (DO), chemical oxygen demand, and biochemical oxygen demand, were examined.

Temperature

Water temperature plays an essential role in all bacteriological studies. The temperature of river water was recorded utilizing a centigrade thermometer at 0.1^0 C at the time of sampling on the study sites. The water samples were collected in a large plastic container and the thermometer was immediately dipped into the water. It was kept steady for about a minute and then the temperature was noted. The temperature was recorded before the samples were fixed for the determination of dissolved oxygen as the solubility of oxygen is dependent on temperature (Aman *et.al.,* 2008)**.**

Transparency

The amount of suspended organic and inorganic debris is directly proportional to the turbidity of the water, which is inversely related to transparency. The turbidity in the lotic water system is usually produced by particles of clay and silt, organic matter, plankton, etc. Various methods are used for the determination of turbidity, for example, the photoelectric method, U.S. Geological Survey turbimeter, Secchi disc, etc. In the present study, Secchi disc was used for the determination of turbidity. The Secchi disc consists of a circular metal plate with a 20 cm diameter and four equally spaced quadrants on its upper surface. Each quadrant is painted black or white alternately in a radial fashion. The lower surface of the metal plate is painted black to eliminate the reflection of light from that surface. The degree of distinction between the two colors in water determines the index of turbidity of water (Mohanakavitha, *et al.,* 2019).

Hydrogen ion concentration (pH)

The pH is the analysis of the intensity of the acidity or the alkalinity and evaluates the concentration of hydrogen ions in water. It was measured by using a Digital Portable Water Analysis Kit (Century CK 711). The pH electrode was cleansed with distilled water and dried. It was then calibrated with a standard solution of pH 4. The collected water sample was poured into a conical flask. The pH electrode was again rinsed with distilled water and dehydrated thoroughly. It was then dipped in the water sample and the pH was recorded (Amuda *et al.,* 2007).

Dissolved Oxygen

For the measurement of dissolved oxygen, the given water sample was immediately fixed at the sampling sites with manganous sulfate and alkali iodide azide. Winkler's iodide azide method was applied to evaluate the dissolved oxygen of river water samples. By precipitating manganic basic oxide, which becomes dispersed by a more concentrated form of H_2SO_4 to generate manganic sulfate, the DO of samples of water was determined. As soon as there is potassium iodide available, it combines with it to release I2, which is then measured by titration with a sodium thiosulphate solution (0.025 N) (Patel, 2020).

The following list of chemical processes used in the method:

$2\ Mn\ (OH)2 + O2 \longrightarrow 2MnO\ (OH)_2$(Brown ppt).

$MnO\ (OH)_2 + 2\ H2SO4 \longrightarrow Mn\ (SO_4)_2 + 3\ H_2O$

$Mn\ (SO4)2 + 2KI \longrightarrow MnSO_4 + K_2SO_4 + I_2$

$Na_2S_2O_3 + I_2 \longrightarrow Na_2S_2O_3 + 2NaI$

The amount of oxygen present in the water samples is equal to the amount of iodine emitted during these reactions. The formula below was used to compute the DO value:

$$Dissolved\ Oxygen\ \left(\frac{mg}{ml}\right) = V \times N \times 8 \times \frac{1000}{Ml\ of\ sample\ used}$$

.

Biological Oxygen Demand (BOD)

Biological Oxygen Demand (BOD) is the amount of dissolved oxygen needed in milligrams per liter for stabilizing the biodegradable organic matter by micro-organisms of the sample under aerobic conditions in a declared time (Patel *et al.,* 2020). BOD of a water sample estimated the difference between the initial DO (Dissolved Oxygen) of the sample and DO after 5 days of incubation of the sample at 20° C in the dark condition in BOD incubator. Diluted water was processed in a glass container by bubbling compressed air in distilled water for around thirty minutes. One ml of each of the following reagents, namely, phosphate buffer solution, magnesium sulphate solution, calcium chloride solution, and ferric chloride solution was added for each litre of diluted water and was mixed thoroughly. The dilution of the sample water was executed according to the expected BOD range. Two sets of BOD bottles were filled. One of them was kept in BOD incubator at 20° C for five days and the DO of another set was determined instantly. Later, the dissolved oxygen concentration was determined after five days of incubation. One set of blanks was also run.

$$Biological\ Oxygen\ demand \frac{mg}{ml} = (D0 - Ds) \times df$$

Chemical Oxygen Demand (COD)

To calculate the COD of water samples, the dichromate reflux method was adopted. COD is the evaluation of the oxygen equivalent of those constituents in the samples that are susceptible to dichromate oxidation in acid conditions. A pre-determined volume of water sample was refluxed with an established volume of potassium dichromate and conc. H_2SO_4 in a reflux flask for two hours. Finally, the whole content of the reflux flask was washed with distilled water and the remaining amount of $K_2Cr_2O_7$ was titrated with ferrous ammonium sulphate using ferroin as an indicator (Kumar & Desai, 2011).

$$Chemical\ oxygen\ demand\ \left(\frac{mg}{ml}\right) = (a-b) \times N \times \frac{8000}{Sample\ used\ in\ ml}$$

Total Hardness

The total hardness of water samples was estimated by "EDTA titrimetric method". The hardness of water is due to the presence of bivalent alkaline earth elements, calcium (Ca^{++}) and magnesium (Mg^{++}). Anions such as carbonate, bicarbonate, sulphate, chloride, nitrate, etc., produce very low hardness. The fact that disodium dihydrogen ethylene diamine tetraacetic acid (Na_2EDTA) designs a slightly iodized colourless stable complex with alkaline earth ions' is used for the determination of hardness. The indicator, Eriochrome Black T is bright blue in the absence of alkaline earth ions but in their presence, it forms a deep red colour complex at pH of 10.0 ± 1. Thus, using Eriochrome Black T as an indicator, the Ca^{+2} and Mg^{+2} ions can be titrated with Na_2EDTA (0.01 M) (Singh, 2020).

$$Total\ Hardness = ml.of\ EDTA\ used/ml\ of\ \ sample\ used\ \times 1000$$

Total Dissolved Solids (TDS)

The dissolved solids stand for the diversified minerals present in water. Total dissolved solids (TDS) were measured by using a Digital Portable Water Analysis Kit (Patel, et al., 2020). (Century CK 711). The cell was cleaned, dried, and connected to the TDS socket. The water sample was poured into a conical flask. The cell was dipped into the water sample and the value of TDS was recorded.

Treatment

Experimental group design

The study conducted by Pandi Prabha and Rajkumar in (2015) determined that the sub-lethal levels of lead in *fish* were 12 parts per million (ppm). To investigate its protective impact, the fish were divided into 5 distinct groups as outlined below:
Animals were placed into five groups of 15 each.

Group I: The fish were exclusively nourished with standard fish feed as control.

Group II: The Lead Treated Group (Pb) was exposed to 10mg/l

Group III, IV, and Group V: treated with 12 mg/l, 24 mg/l, 26 mg/l.

Sample collection

The samples of *Channa punctata* species of fish, along with samples of water, were collected from the treated sample on 5 that day 10 days after exposure. The water and sample sediments were separately gathered in transparent cleaned plastic bottles. The sediments underwent desiccation in an oven at a temperature of 100°C for 24 hours. The fish sample was separately stored in vials filled with a 5 % strength solution of formalin. To prevent deterioration, the samples were safeguarded using cleaned polythene bags and kept at a temperature of -20°C in a deep refrigerator until they were prepared for subsequent examination. The fish samples were dissected into separate pieces, including the anatomical features of a fish including the head, tails, lower abdomen, scales, fins, as well as gills, using stainless steel blades that are resistant to corrosion. Every single sample was set aside in a

separate porcelain dish and thereafter transferred to the oven for drying at an oven temperature of 100°C for 24 hours.

The desiccated specimens were pulverized into tiny fragments. Every specimen underwent treatment with a volume of 10 milliliters of highly concentrated (HNO_3) and 2 milliliters of (H_2O_2), with approximately 2.0 grams of each sample undergoing this treatment. The specimens underwent digestion utilizing a Jane Way Model-1000 hot plate, and 6.0 g of each sample was extracted for total lipids following the standard protocol.

Blood Parameters

The blood samples were taken, and hematological analysis was performed right away. The blood had been diluted using the proper diluting fluids, and the improved Neubauer hemocytometer was used to calculate the RBC and WBC counts. Each blood sample had its counts repeated. To calculate hemoglobin (HB) percentage (HB %), Sahli's hemoglobin meter was utilized. Using micro hematocrit capillaries filled with blood and centrifuged at 8,700×g for 5 minutes, hemoglobin (HCT) was calculated and expressed as a percentage of total blood volume. Mean corpuscular volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated from the average values of HB%. The average values of HB% were used to determine mean corpuscular volume (MCV), means cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) (Esmaeili, N., 2021).

Biochemical analysis

The concentrations of 8-Hydroxydeoxyguanosine in the fish were measured using an ELISA kit, which utilizes. The absorption value at 450 nm was measured using an ELISA reader. The levels of 8-OHdG have been established by extrapolating from a pre-existing curve and are expressed as nanograms per milligram of protein (Seibel *et al.,* 2021).

Gene expression

The quantification of gene expression associated with programmed cell death in liver tissue was evaluated using real-time polymerase chain reaction. The tissue from the liver was utilized to isolate total RNA employing an RNA Isolation Kit, according to the instructions

supplied by the product's manufacturer. The RNA quantity was quantified utilizing a Nanodrop 2000 spectrophotometer, whereas the RNA purity was assessed utilizing 1% agarose gel in electrophoresis (Seibel *et al.,* 2021). The reverse transcription process was carried out following the instructions provided by the manufacturer. Primer sequences were constructed for replicating cDNA of the genes p53, bax, bcl-2, apaf-1, and caspase-3. The design was based on sequences of genes accessible on NCBI (Table 1).

Table 3.2 Summarization of primers used to amplify the genes.

S.No.	Gene	Sequences
1.	P^{53}	F'CTTATTGGACCGGAGA R'GTGATGGCATCCCAAAGAGC
2.	bax	F'AGGAGGTGATCAAGGCAAAAGT R'TCCATGCCTTTTAACCCGCT
3.	Bcl-2	F'TGCAAAGAGGTGGTCAAGACG R'TCCACAAAGGCATCCCATCCT
4.	Apaf-1	F'AGGGAGAAACTCTACCGGCT R'CTCCAGGGAAGCACTCTTCG
5.	Caspase 3	F'TCCACAGCTCCAGGCTACTA R'TGAAGCTCCACGTCTTTCCC

Optical Microscopy

Light microscopy histological methods are generally followed by Caill-Milly (2023). The samples were first rinsed in tap water, followed by several applications of alcohols for dehydration before being submerged in an xylene bath for intermediary impregnation. This procedure was repeated after a 48-hour fixation period in Bouin's solution.

For impregnation, samples were maintained overnight in liquid paraffin at 56 ^{o}C in an incubator. Then, using Leuckart's bars as a mold, samples will be embedded in paraffin to Make solid blocks with the tissue samples in them. Following solidification, slices of 5-7 m thickness were cut from the tissue samples inside the blocks using a microtome. To prevent a rough surface, slices will be placed in a container with warm water. A solution of albumin was used to help the tissue slices adhere to the slides. Using xylene as a solvent, paraffin

was removed from slides before being treated with a graduated sequence of alcohols and taking a bath in demineralized water. Hematoxylin and eosine, which give cells' nuclei a violet colour and their cytoplasm and intercellular materials a reddish-orange color, respectively, will be used to stain the slides. Dehydrating the slides with alcohols 95° and 100° and xylene will then is done. To differentiate the mucous in the gills and gut, Alcian blue 8GX at pH=2.5 was used with Nuclear Fast Redas as a counterstain. Leica Microsystems' DMLB model DMLB image system was used with an optical microscope to conduct the histology observations.

Determination of Total Protein

The method outlined by Bradford (1976) was used to determine the concentration of total protein. This technique depends on Coomassie Blue G-250, an acidic dye, attaching to proteins to change it into a blue state that OD at 595 nm and enables spectrophotometric estimation of the protein concentration.

Determination of Enzymatic Activity

The spectrophotometric approach which follows the decrease in 240 nm absorbance brought on by H_2O_2 intake, was used to assess CAT activity. The method developed by Habig *et al.,* (1974) was used to evaluate GST activity. This method involves monitoring the production of the measuring the activity of the enzyme using a compound of glutathione (GSH) at 340 nm. The nitroblue tetrazolium (NBT) method, which was developed by Sun *et al.,* (1988), was used to determine SOD activity. In this procedure, xanthine-oxidase (XOD) produces superoxide radical (O^{2-}), which reduces NBT to formazan, which may be measured at 560 nm.

Statistical analysis

One-way ANOVA was used for statistical analysis of the results after the data have been evaluated for normality and homogeneity (using Leven's test) and, if necessary, correctly converted.

Chapter-4

RESULTS

4. Temperatures and Turbidity

The temperature of Gomti river water was in the range of 20-30°C. The highest temperature was observed 30°C at site Vaikunth dham and lowest 20°C at Dali Ganj site. Comparatively higher temperatures at places could be attributed to the declined flow rate or very shallow Rocky River bed exposed to direct sunlight raising the temperature of water flowing through/over. So, temperature of the river water was detected to be a function of depth, turbulence, time of the day, and heat input from outer environment.

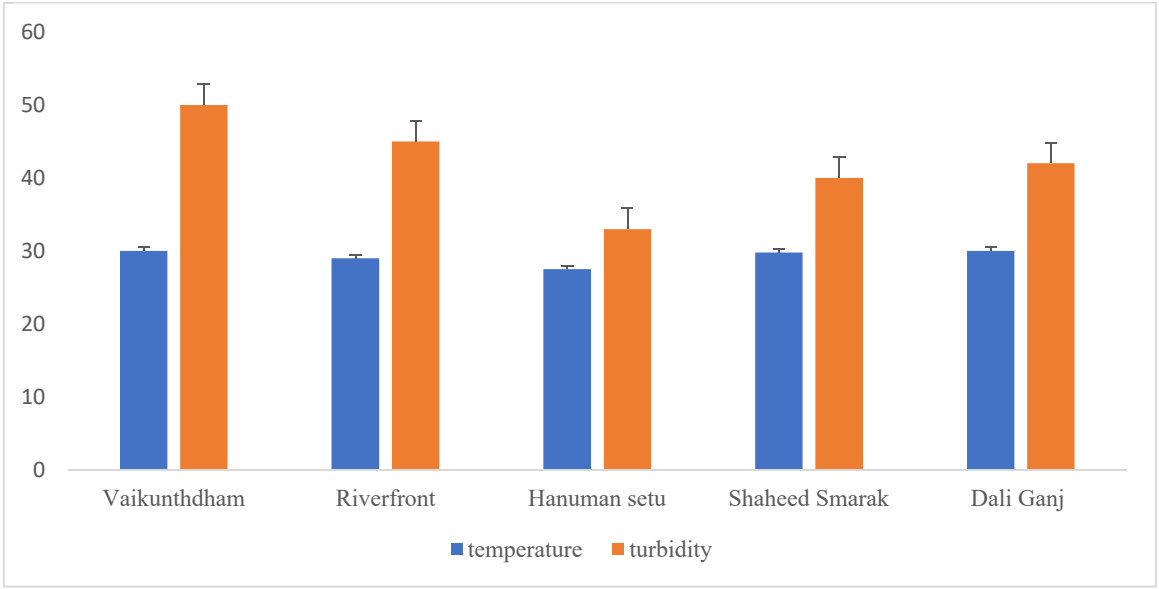

Figure 4.1 Temperature and Turbidity analysis of collected water samples

Physicochemical Analysis of collected Water Samples

Table 4.1 Chemical analysis of collected water samples of Gomti River

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
BOD	19.33±2.08	14.33±2.08	22.02± 2.65	24.67±4.51	21.67±2.08
COD	327.1±8.19	245.01±5	259.67±1.53	358.67±9.61	233±18.01
Hardness	118±7.21	133.67±3.21	148.33±9.07	176±8.57	156.67±2.31
TDS	312.33±2.52	437.67±2.52	431.33±10.02	308.33±7.64	432±15.72
pH	6.13±0.32	5.01±1	6.87±0.32	6.13±0.59	7.33±0.29
Lead (Pb)	10 ppm	12 ppm	30 ppm	26 ppm	24 ppm

An evaluation of pH value in relation to elementary water quality parameters established by the Environment Protection Act of 1986 in terms of pH requirements for class-water in the range of 5±0.14 to 7.33±0.058. The pH change in the river along the Lucknow stretch is within normal ranges, and the river water quality is unaffected by pH.

BOD was also determined of collected sample and it was found that Higher values of BOD suggest the release of decomposing organic matter into the river via an industrial effluents stream that may also be responsible for the drop in DO level. B.O.D. of Class-A waters should not exceed 2.0 mg/l, according to regulations. In contrast, consistently high BOD levels in river water suggest significant organic pollution. The B.O.D. value at Shaheed Smarak sample 4 (S-4) appears to be at its maximum (24.67±0.43 mg/l), indicating that there is the most outflow of bio-degradable organic matter at this site.

The COD data indicates the value variation. The COD for sewage and trade effluent discharge into streams must be less than 250 mg/l. Although the results were determined to be above the guidelines, care must be taken to manage the pollutant load entering the river. At Shaheed Smarak (S-4), the COD appears to be at its highest (358.67±0.19), indicating that sewage disposal is above and beyond the allowed limit.

The results depicted that the hardness content varies dramatically at several sampling locations. Even if slight variations in time and space are obvious. The dumping of industrial and municipal effluent into the river is the most common cause of such changes. A larger concentration of hardness appears in Shaheed Smarak (176±0.16mg/L) and Dali Ganj (156.67 ±0.19 mg/L), which usually represents major industrial and municipal discharge at these locations.

Total dissolved solids values obtained at several sampling sites showed that the Total Dissolved Solids values have a peaking tendency in riverfront (S-2) (437.67±0.12 ppm), hanuman setu (431.33±0.04 ppm), this also demonstrates the influence of industrial wastewater dumped into the river on vegetation and fauna. Lead heavy metal was also analyzed at selected site and maximum lead content was obtained at site III and minimum were obtained at site I.

4.1. Water parameter analysis of treated aquarium

The experiment's data revealed that there was no appreciable difference in pH or temperature between the treatment and control groups. The amount of dissolved oxygen in the water decreased steadily over time in all treatment groups, as seen by data gathered after exposure to heavy metals for five and ten days. Comparing the Control group to the other treatment groups, there was a little decrease in dissolved oxygen. In the treated group, 26 milligrams per liter had high dissolved oxygen while 10 milligrams per liter had low dissolved oxygen. Moreover, the findings demonstrated that while ammonia levels decreased as heavy metal (Pb) exposure dosages (10-26 milligrams per liter) increased, nitrate and nitrite levels climbed steadily. The nitrate contents in the five- and ten-day control groups were the same, at 9.5 ppm. After being exposed to 10 mg/liter, 12 mg/liter, 24 mg/liter, and 26 mg/liter for ten days, the nitrate levels were 18.3ppm, 42.1ppm, 33.8ppm, and 48.6ppm, respectively. Likewise, following five days of exposure to 10 mg/liter, 12 mg/liter, 24 mg/liter, and 26 mg/liter, the nitrate levels were 16.6ppm, 39.2ppm, 40ppm, and 47ppm correspondingly.

Additionally, the control groups' nitrite levels were 3.8ppm and 7.8ppm after 10 and 5 days, respectively. Nitrite levels were 4 ppm , 4.6ppm, 3.6 ppm , and 4.4ppm after a 10-day exposure to 26milligrams per liter, 24milligrams per liter, 12milligrams per liter, and 10milligrams per liter; levels were 8.5 ppm , 9.ppm1, 7.6ppm, and 8.8 ppm after a 5-day

exposure to 10milligrams per liter, 12milligrams per liter, 24milligrams per liter, and 26milligrams per liter.

Furthermore, the control groups' ammonia levels were 0.2 and 0.4 on days 5 and 10, respectively. Ammonia levels were 0.4 ppm, 0.1ppm, 0.3ppm, and 0.34ppm on the tenth day of exposure to 26 milligrams per liter, 24 milligrams per liter, 12 milligrams per liter, and 10 milligrams per liter of titanium oxide heavy metal exposure; on the fifth day of exposure, the ammonia levels were 0.3 ppm , 0.4 ppm, 0.3ppm, and 0.2 ppm.

Behavior studies of *Channa punctatus*:

To investigate the environmental toxicity of heavy metals to fish, behavior studies were conducted. The results of the research revealed that all groups of fish treated with mercury exhibited surface vertical swimming, jerky movement, stand-still zigzag movement, and uncoordinated movement.

4.2. Heavy metal consequences for the body weight, organ weight, and hepatosomatic index in the *Channa punctatus*

The fish's body weight and organ weight were measured in the control group. The results indicated that the weight of the eyes, spleen, liver, Gills, and intestines were 33±15.24 g, 0.043±0.018 g, 0.2036±0.153 g, 0.3854±0.283 g, 0.3234±0.11503 g, and 85.78±191.30 g, respectively. Furthermore, measurements were taken of the overall length, kidney length, and hepatosomatic index, which were 15.7±2.38 cm, 0.12314±0.084 cm, and 0.616, respectively. The statistical significance of the data was $p<0.05$.

10 milligrams per liter

The weight of the fish's body and organs were measured in the treated group of 10 fish. The data obtained from this observation suggested that the treated group's weight increased, coming in at 34±8.21g, 0.043±0.018 g, 0.093±0.13 g, 0.23±0.069g, 0.49 ±0.20g, and 0.26±0.07g, respectively, for the spleen, liver, eyes, and intestine. Furthermore, measurements of 14.9±1.710 cm for total length and 0.103±0.05 cm for kidney length were also made. There was statistical significance in the outcomes. The body weight and organ weight of the 10 treated fish were also observed over a period of 10 days. The data obtained from this observation indicated that the

body weight, spleen weight, liver weight, Gills weight, intestine weight, and eyes weight of the treated group were 38±8.3g, 0.028±0.008g, 0.36±0.19g, 0.348 ±0.111g, 0.224 ±0.120g, and 0.33±11 g, respectively. Furthermore, measurements of overall length and kidney length were made, with results of 14.6±2.30 cm and 0.096±0.0207 cm, respectively. Statistics suggested that the results were significant.

12 milligrams per liter

The treated group's body weight, spleen weight, liver weight, Gills weight, intestine weight, and eyes weight were found to be 35.8±6.57g, 0.040±0.04 g, 0.23±0.05 g, 0.382±0.240g, 0.201 ±0.049g, and 0.32±0.06g, respectively, based on observations made of the fish's weight. Furthermore, measurements were taken of the kidney and overall length, which were 15.86±0.98 cm and 0.14±0.094 cm, respectively. The results were statistically significant. The treated group fish were observed to have body weight and organ weight of 12 milligrams per liter for ten days. The data obtained from this observation suggested that the body weight, spleen weight, liver weight, Gills weight, intestine weight, and eyes weight of the treated group were 47±4.47g, 0.03±0.01 g, 0.406±0.09 g, 0.372 ±0.06g, 0.216 ±0.042g, and 0.32±0.06 g, respectively. Furthermore, measurements were taken of the kidney and total length, which came out to be 15±0.79 cm and 0.136±0.028 cm, respectively. The results were statistically significant.

24 milligrams per liter

The treated group fish were observed to have body weight and organ weight of 24. The data obtained from this observation suggested that the body weight, spleen weight, liver weight, Gills weight, intestine weight, and eyes weight of the treated group were 34±5.47g, 0.040±0. 0.04 g, 0.22±0.04g, 1.01 ±1.30g, 0.40 ±0.15g, and 0.33±0.05g, respectively. Furthermore, measurements were taken of the kidney and total length, which came out to be 15.96±1.08 cm and 0.09±0.04 cm, respectively. According to statistics, the results were remarkable. Comparably, the body weight and organ weight of the fish were measured in the treated group fish with 24 milligrams per liter for 10 days. The data obtained from this indicated that the body weight, spleen weight, liver weight, Gills weight, intestine weight, and eyes weight of the treated group were 41±8.9g, 0.22±0.110g, 0.302 ±0.088g, 0.384 ±0.11g, 0.214±0.06, and 0.358±0.08 g, respectively. Furthermore, measurements of 15.2±1.30 cm for overall length and 0.302±0.088 cm for kidney length were made. Statistics illustrated how important the results were.

26 milligrams per liter

The weight of the fish's body and organs were measured in the treated group of 26 fish. The results of this observation indicated that the weight of the treated group's spleen, liver, intestine, eyes, and Gills were 30.4±7.12g, 0.014±0.008g, 0.260±0.055g, 0.352±0.128g, 0.288±0.078g, and 49.36±109.7 g, respectively. Furthermore, measurements of 14.5±1.54 cm for total length and 0.08±0.034 cm for kidney length were made. Statistics suggested that the outcomes were noteworthy. The results appeared statistically significant. Similarly, the body weight and organ weight of the fish were observed in the treated group fish with 26 milligrams per liter for 10 days. Data observed from this suggested that the body weight, spleen weight, liver weight, Gills weight, intestine weight, and eyes weight of the treated group fish were 38±8.3g, 0.026±0.008g, 0.372 ±0.06g, 0.41±0.08g, 0.188±034, 0.31±0.02,g respectively. In addition, total length and kidney length were also measured which were 15.14±1.05cm, and 0.532±0.38 cm respectively was observed. Statistics suggested that the results were relevant.

Table 4.2 Effects of Heavy metal on the body weight, organ weight, and hepatosomatic index in the fish, *Channa punctatus.* Values are expressed as Manisem (One Way ANOVA). The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P < 0.05$).

Heavy metal										
Parameters	**Day-5**					**Day-10**				
	Control	**10mg**	**12mg**	**24mg**	**26mg**	**Control**	**10mg**	**12mg**	**24mg**	**26mg**
Body weight (g)	33 ± 15.2	34 ± 8.2	35.8 ± 6.5	34 ± 5.4	30.4 ± 7.1	37±8.36	38±8.3	47±4.47	41±8.94	38±8.36
Fish length (cm)	15.7 ± 2.3	14.9 ± 1.7	15.86 ± 0.98	15.96 ± 1.08	14.5 ± 1.54	15.9±1.74	14.6±2.3	15±0.79	15.2±1.3	15.14±1.05
Gill weight (g)	0.38±0.28	0.49±0.20	0.38±0.24	1.01±1.30	0.35±0.12	0.328±0.15	0.34±0.11	0.372±0.06	0.38±0.11	0.41±0.08
Hepatosomatic index	0.616	0.691	0.662	0.674	0.855	1.086	0.947	0.864	0.737	0.979
Spleen weight	0.043±0.02	0.09±0.14	0.04±0.04	0.04±0.04	0.01±0.01	0.032±0.01	0.028±0.01	0.033±0.01	0.022±0.01	0.026±0.01
Kidney weight	0.123±0.08	0.103±0.05	0.14±0.09	0.09±0.04	0.08±0.03	0.12±0.04	0.096±0.02	0.136±0.02	0.302±0.08	0.532±0.38
Intestine	0.32±0.11	0.29±0.07	0.20±0.04	0.40±0.15	0.28±0.07	0.27±0.14	0.224±0.12	0.216±0.04	0.214±0.065	0.188±0.03
Eye	0.26±0.09	0.26±0.07	0.32±0.06	0.33±0.05	0.27±0.07	0.36±0.07	0.33±0.116	0.32±0.06	0.358±0.08	0.31±0.029
Liver	0.203±0.153	0.235±0.06	0.237±0.05	0.229±0.04	0.26±0.05	0.402±0.19	0.36±0.19	0.406±0.09	0.302±0.08	0.372±0.06

4.3. Histopathological observation of *Channa punctatus* Liver

4.3.1. Histopathology of liver on treatment with Heavy metal on day 5

In control fish liver tissue, the portal vein's histological structure was normal, showing sinusoids, homogenous cytoplasm, and hepatocytes in the hepatic parenchyma. A histological analysis of the liver was conducted after five days of exposure to a heavy metal dose of 10 mg/liter. The obtained results clarified the observations of blood congestion, cytoplasmic vacuolation, patchy degeneration, melano-macrophage centers, patchy central vein, and hyperplasia with exposure to 10 milligrams per liter of pyknotic nuclei.

A histological analysis of the liver was conducted after five days of exposure to a heavy metal dose of 12 milligrams per liter. The obtained results clarified the observations of cytoplasmic vacuolation, mononuclear cell infiltration, fatty alterations, and hyperemia during exposure to 12 milligrams per liter of melano-macrophage centers.

After five days of exposure to a heavy metal dose of 24 mg/liter, a histological analysis of the liver was conducted. The obtained results clarified the reported fatty alterations, hyperemia, cytoplasmic vacuolation, infiltration of mononuclear cells, and exposure to 24 mg/liter of macrophage centers.

After five days of exposure to the 26 mg/liter doses of heavy metals, a liver histological test was conducted. The obtained data clarified that after 26 mg/liter of exposure. Hepatic vein degeneration, damaged hepatic lobule, damaged hepatocytes, fragmented kuffer cell, damaged central vein, pyrcnotic nuclei, and cytoplasmic vacuolation were also noted.

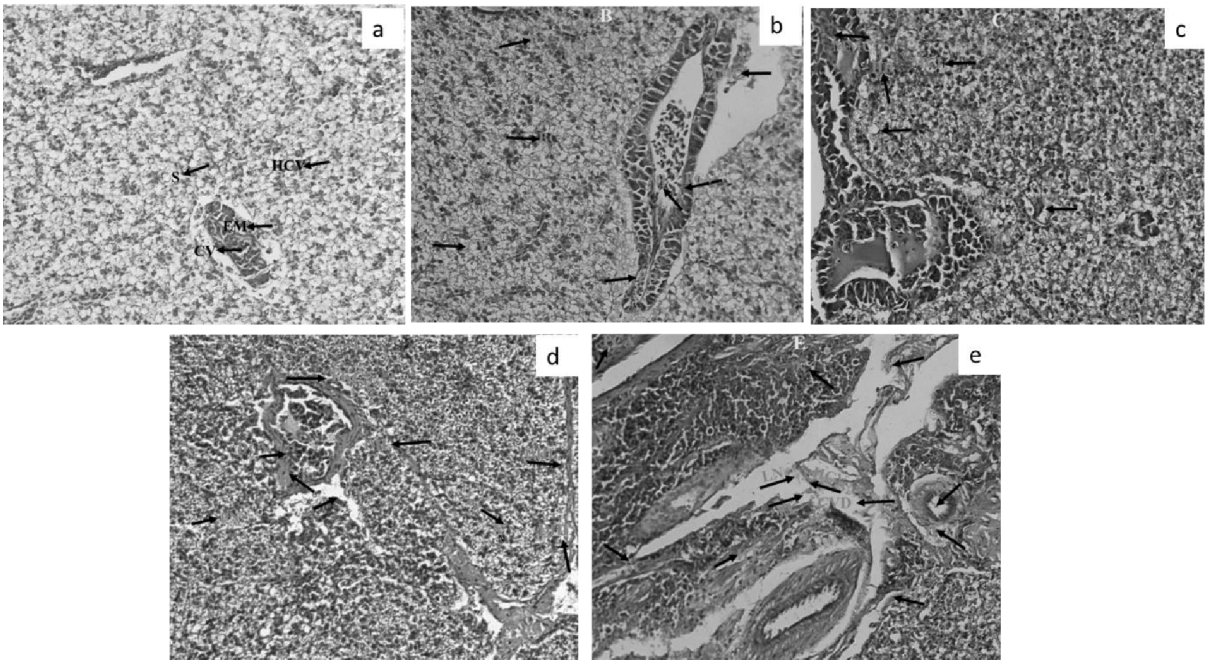

Figure 4.2 *C. punctatus* liver section observations at 5 days, H&E at 100X. Control fish (a); Fish subjected to various amounts of Heavy metal (b, 26milligrams per liter; c, 24milligrams per liter; d, 12milligrams per liter; e, 10milligrams per liter).

S: Sinusoids, **HCV:** Hepatocyte with central nuclei, **EM:** Epithelium membrane; **CV:** Central vain, **MMC:** Melano-macrophage centres, **PCV:** Patchy Central vain, **H:** Hyperplasia, **PN:** pyknotic nuclei, **CV:** cytoplasmic vacuolation, **PD:** Patchy degeneration, **BC:** Blood congestion, **MCI:** mononuclear cells infiltration, **MMC:** Melano-macrophage centres; **HR:** Hyperaemia, **FKC:** Fragmented kuffer cell, **DCV:** Damaged central vain, **DH:** Damaged hepatocytes, **DHV:** Degeneration of hepatic veins, **N:** necrosis; **DHL:** Damaged hepatic lobule,

H: Hyperemia, **DHA:** Damaged hepatic artery; **CVD:** Central vain disappear; **HCV:** Huge cytoplasmic vacuolation, **VD:** Veins disappear, **HT:** Hypertrophy, **EI:** Eosinophilic infiltration, **LN:** Loss of nuclei of hepatocyte; **SC:** Sinusoidal congestion; **PCA:** portal canal absent.

Histopathology of liver on treatment with Heavy metal on day 10 day

The 10milligrams per liter doses of heavy metals were exposed for 10 days, and a histopathological examination of the liver was observed. Obtained results explained that on exposure to 10milligrams per liter. Mononuclear cell infiltration, Accumulation of kuffer, congested blood vessels, Hyperemia, and Fatty changes were observed.

The 12 milligrams per liter doses of heavy metals were exposed for 10 days, and a histopathological examination of the liver was observed. Obtained results explained that on exposure to 12 milligrams per liter. Mononuclear cell infiltration, Accumulation of kuffer, congested blood vessels, Hyperemia, and Fatty changes were observed.

The 24 milligrams per liter doses of heavy metals were exposed for 10 days, and a histopathological examination of the liver was observed. Obtained results explained that on exposure of 24 milligrams per liter. Damaged hepatocytes, Cytoplasmic vacuolation, Pycnotic Nuclei, Dilated sinusoids, Accumulation of kuffer cells, and damaged central vain were observed.

The 26 mg/liter doses of heavy metals were exposed for 10 days, and a histopathological examination of the liver was observed. Obtained results explained that on exposure to 26 milligrams per liter. Cytoplasmic Vaculolation, Hyperemia, Melano-macrophage centers, Fatty changes, congested blood vessels, Dilated sinusoids, Accumulation of kuffer cells, mononuclear cells infiltration were observed.

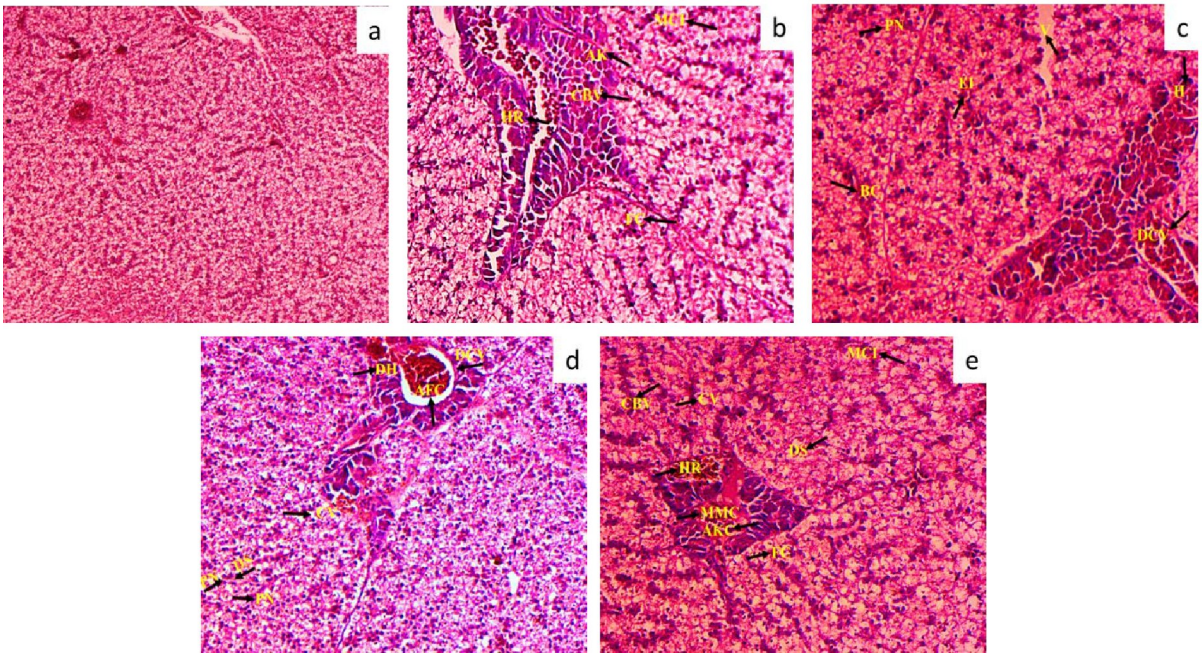

Figure 4.3 *C. punctatus* liver section observations at 10 days, H&E at 100X. Control fish (a); Fish subjected to various amounts of Heavy metal (b, 10milligrams per liter; c, 12milligrams per liter; d, 24milligrams per liter; e, 26milligrams per liter).

MCI: mononuclear cells infiltration; **AK:** Accumulation of kupffer; **CBV:** congested blood vessel; **HR:** Hyperemia; **FC**: Fatty changes**, BC:** Blood Congestion; **V:** Vacuolation; **EI:** Erythrocyte infiltration into blood sinusoids, **DCV:** Damaged Central vain, **PN:** pyknotic nuclei, **DH**: Damaged hepatocytes, **DS:** Dilated sinusoids **CV:** Cytoplasmic vacuolation **AFC:** Accumulation of kuffer cells, **DS:** Dilated Sinusoids, **MMC:** Melano macrophage centres, **FC:** Fatty changes **AKC:** Accumulation of kuffer cells.

4.4 Gills

4.41 Histopathological examination gills on treatment with Heavy metalon day 5.

The healthy fish's normal gills revealed a clean arrangement of primary as well as secondary lamellae that could be easily distinguished. The 10 mg/mL dose of heavy metal was exposed for 5 days, and histopathological examination of gills was observed. Obtained results explained that on exposure of 10 mg/mL Interlamellar hyperplasia with an almost total fusion of secondary lamellae, Detachment of epithelium layer in the secondary lamella, aneurysm, partial fusion, hyperplasia of secondary lamellae was observed.

The 12 mg/mL dose of heavy metal was exposed for 5 days, and histopathological examination of gills was observed. Obtained results explained that on exposure of 12 mg/mL Detachment of the epithelium layer in the secondary lamella, mononuclear leukocyte infiltrates, telangiectasia, Hyperplasia, and Erythrocyte infiltration alterations were observed.

The 24 mg/mL dose of heavy metal was exposed for 5 days, and histopathological examination of gills was observed. Obtained results explained that on exposure of 24 mg/mL dilatation of the venous sinus, coalescent interlamellar hyperplasia, Partial fusion, hyperplasia of secondary lamellae, Detachment of epithelium layer in the secondary lamella, Telangiectasis, Hyperplasia was observed.

The 26 mg/mL dose of heavy metal was exposed for 5 days, and histopathological examination of gills was observed. Obtained results explained that on exposure of 26 mg/mL congestion of the central vein, interlamellar hyperplasia, telangiectasia, Detachment of cells, Erythrocyte infiltration, interlamellar hyperplasia with an almost total fusion of secondary lamellae Hyperplasia was observed.

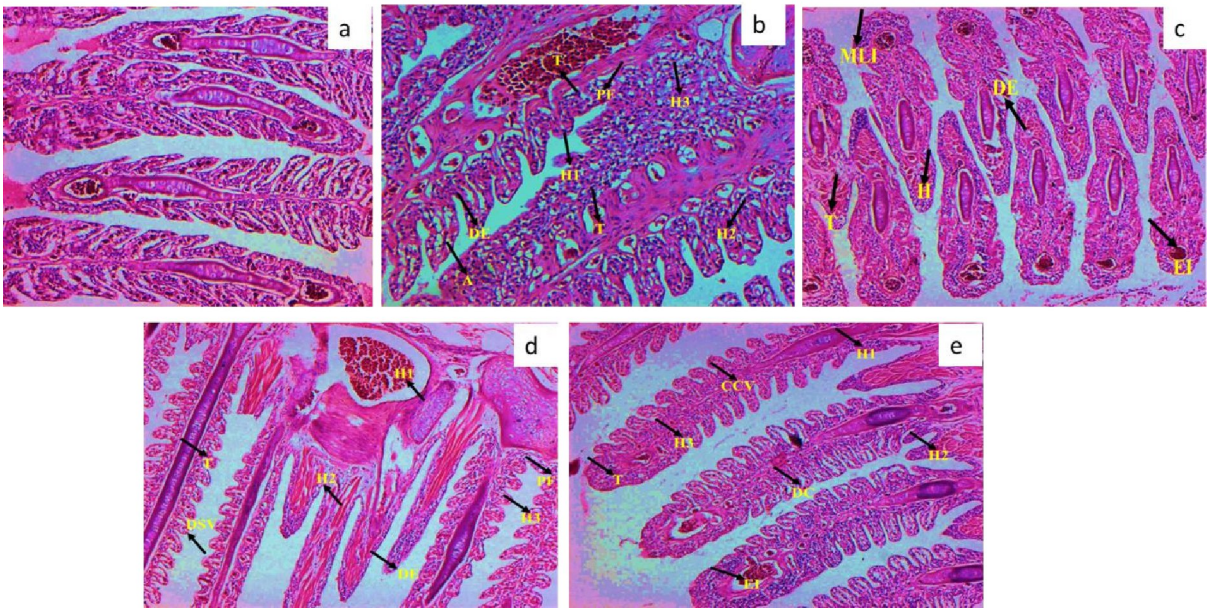

Figure 4.4 *C. punctatus* gills section observations at 5 days, H&E at 100X. Control fish (a); Different concentrations of Heavy metal exposed fishes (b, 10 milligrams per liter; c, 12 milligrams per liter; d, 24 milligrams per liter; e, 26 milligrams per liter).

H1: Interlamellar hyperplasia with almost total fusion of secondary lamellae, **DE:** Detachment of epithelium layer in the secondary lamella, **A:** aneurysm, **H3:** coalescent interlamellar hyperplasia, **PF:** Partial fusion, **H2:** hyperplasia of secondary lamellae, **T:** Revealing telangiectasis at the tips, **MLI:** mononuclear leukocyte infiltrate, **T:** telangiectasia, **H:** Hyperplasia, **EI:** Erythrocyte infiltration, **DSV:** dilatation of the venous sinus, **DE:** Detachment of epithelium layer in the secondary lamella, **CCV:** congestion of the central vein, **DC:** Detachment of cell.

4.4.2 Histopathology of liver on treatment with Titanium oxide Heavy metalson day 10

The 10milligrams per liter dose of heavy metal was exposed for 10 days, and a histopathological examination of the liver was observed. Obtained results explained that on exposure of 10secondary and primary lamellae were thick, initial Aneurysm, complete fusion of several lamellae, Parasitic cyst, Lifting of the respiratory epithelium, The primary as well as the secondary lamellae were seen to club together. The 12milligrams per literdose of heavy metal was exposed for 10 days, and histopathological examination of gills was observed. Obtained results explained that on exposure of 12 the Thickening of the primary and secondary lamellae, coalescent interlamellar hyperplasia, Detachment of cells, was observed.

The 24 milligrams per liter doses of heavy metals were exposed for 10 days, and a histopathological examination of the gills was observed. Obtained results explained that on exposure of 24milligrams per liter the Parasitic cyst, Aneurysm, and Lifting of the respiratory epithelium, complete fusion of several lamellae were observed.

The 26milligrams per liter doses of heavy metals were exposed for 10 days, and a histopathological examination of the gills was observed. Obtained results explained that on exposure of 26milligrams per liter the Thickening of the primary and secondary lamellae, coalescent interlamellar hyperplasia, Revealing telangiectasis at the tips, Partial fusion, was observed.

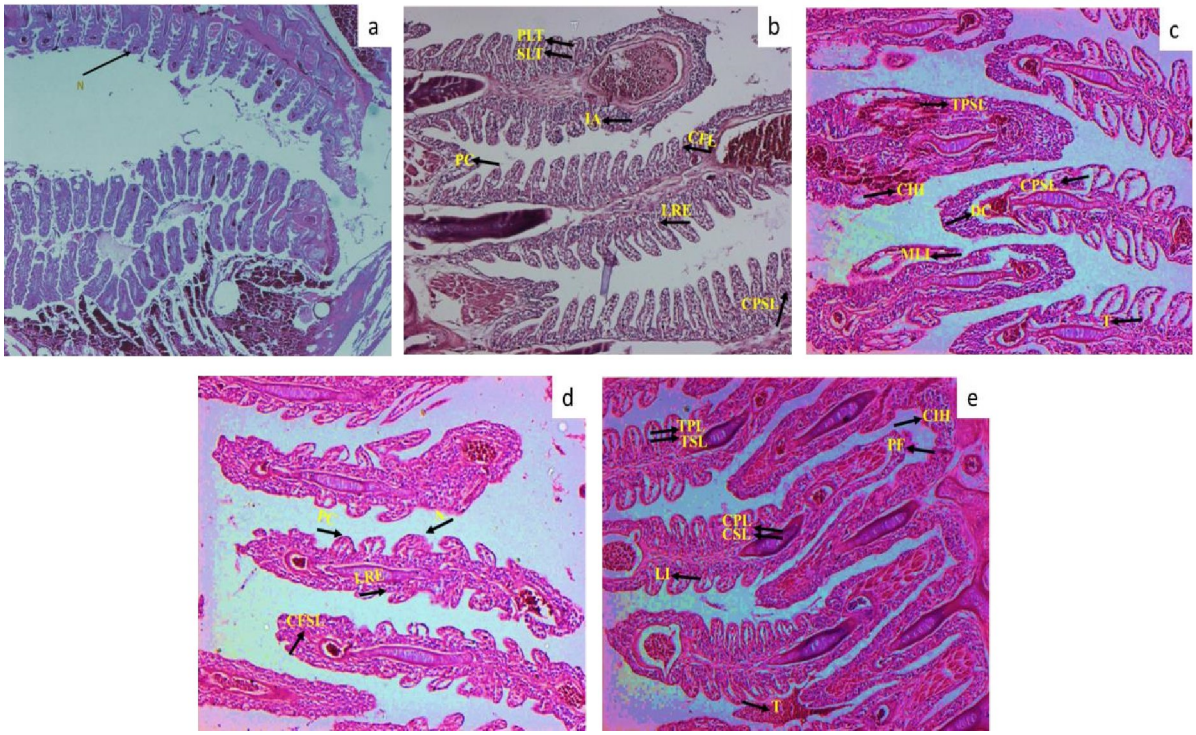

Figure 4.5 *C. punctatus* gills section observations at 10 days, H&E at 100X. Control fish (a); Different concentrations of Heavy metal exposed fishes (b, 10 milligrams per liter; c, 12 milligrams per liter; d, 24 milligrams per liter; e, 26milligrams per liter).

PLT and SLT: secondary and primary lamellae are thick, **IA:** initial Aneurysm, **CFL:** complete fusion of several lamellae **PC:** Parasitic cyst, **LRE:** Lifting of the respiratory epithelium, **CPSL:** main and intermediate lamellae clubbed together **MLI:**a modest invasion of lymphocytes, **T:** Telangiectasi **DC:** Detachment of cell, **CIH:** coalescent interlamellar Hyperplasia **TPSL:** Secondary as well as primary lamellae thickening, **PC:** Parasitic cyst, **A:**Aneurysm**, CFSL:** complete fusion of several lamellae, **TPL:** main and secondary lamellae thickness increase**, T:**telangiectasis at the tips, **LI:** lymphocytic infiltration**, CPL:** Clubbing of primary lamellae, **CSL:** Clubbing of secondary lamellae, **PF:** Partial fusion

4.5 Kidney

4.5.1 Histopathological examination kidney on treatment with Heavy metalon day 5.

In Kidney, normal kuffer cells, glomerulus, bowmen capsuls were obsrvred. The 10 dose of heavy metal was exposed for 5 days, and a histopathological examination of the kidney was

observed. Obtained results explained that on exposure to 10 mg/mL Epithelial cell atrophy, reduced glomeruli cells, Loss of tubular cell components, Large collecting duct, Hydrobic degeneration, Dilated tubule, Patchy degeneration was observed.

The 12milligrams per liter dose of heavy metal was exposed for 5 days, and a histopathological examination of the kidney was observed. Obtained results explained that on exposure of 12 mg/mL Detached epithelium from the basal lamina, Deformation of the Bowman space, Space of bowman capsule, Erythrocyte in glomerular capillaries, Degeneration of epithelium With erythrocyte, Epithelial cell atrophy, Hydrobic degeneration, Renal tubule become degenerate, Visceral membrane damage, Mesangial cell was observed.

The 24milligrams per liter dose of heavy metal was exposed for 5 days, and a histopathological examination of the kidney was observed. Obtained results explained that on exposure to 24 Damaged Glomerulus, Loss of first proximal tubule, Melanomacrophage, collecting duct, Space in women capsules, Mononuclear cells infiltration, Red blood cells, Visceral membrane damage, Nuclear pyknosis, Patchy degeneration was observed.

The 26milligrams per liter dose of heavy metal was exposed for 5 days, and a histopathological examination of the kidney was observed. Obtained results explained that on exposure of 26milligrams per liter Detached epithelial cells from the basal lamina, degenerated renal corpuscle, Proximal convoluted tubule shrunken, tubular cell Components disappeared, and Damaged podocyte cells were observed.

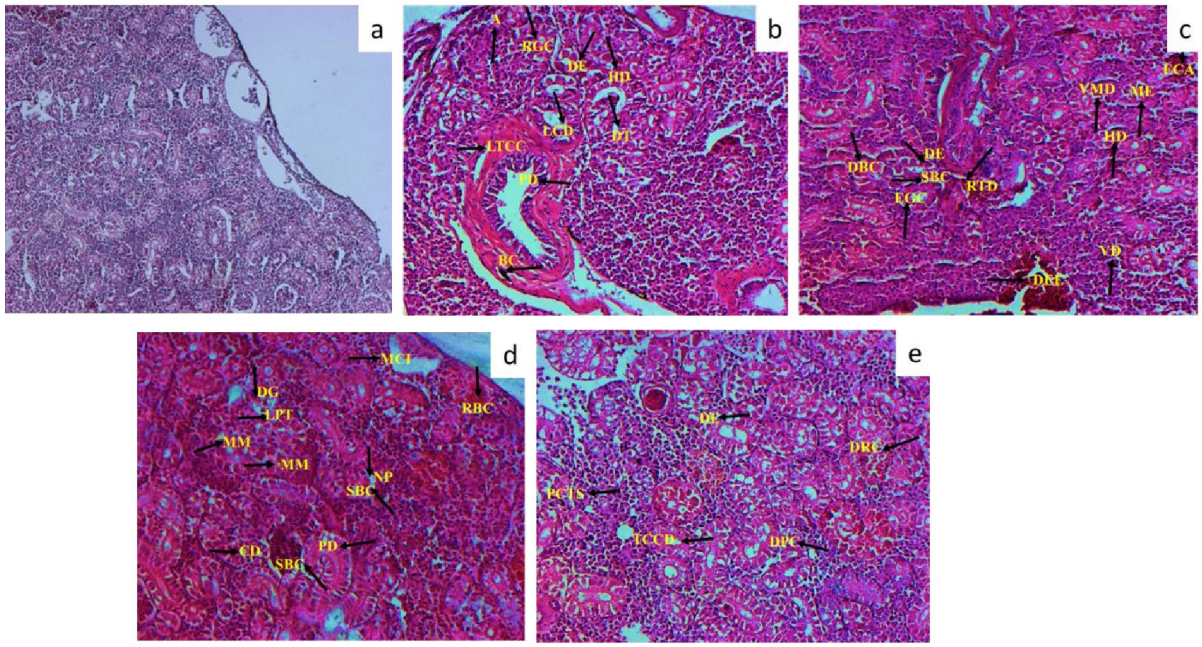

Figure 4.6 *C. punctatus* kidney section observations at 5 days, H&E at 100X. Control fish (a); Different concentrations of Heavy metal exposed fishes (b, 10 milligrams per liter; c, 12milligrams per liter; d, 24 milligrams per liter; e, 26 milligrams per liter).

A: atrophy of epithelial cell, **RGC:** reduced glomeruli cells, **LTCC:** Loss of tubular cell components, **LCD:** Large collecting duct, **BC:**in close proximity to injured renal tubules, a basophilic cluster,**DE:**basal lamina-derived epithelial cells separated, **HD:** Hydrobic degeneration, **DT:** Dilated tubule, **PD:** Patchy degeneration, **DE:** Detached epithelium form basal lamina, **DBC:** Deformation of the Bowman space, **SBC:** Space of bowman capsule, **EGC**: Erythrocyte in glomerular capillaries, **DEE:** Degeneration of epithelium With erythrocyte, **ECA:** Epithelial cell atrophy, **HD:** Hydrobic degeneration, **RTD:** Renal tubule become degenerate, **VMD:** Visceral membrane damage, **VD:** Vacular degeneration, **MC:** Mesangial cell, **DG:** Damaged Glomerulus**, LPT:** Loss of proximal tubule, **MM:** Melanomacrophage, **CD:** Collecting duct, **SBC:** Space in bowman's capsules, **RBC:** Red blood cells, **VMD:** Visceral membrane Damage, **MCI:** Mononuclear cells infiltration, **PD:** Patchy degeneration, **NP:** Nuclear pyknosis **DPC:** Damaged podocyte cell, **TCCD:** tubular cell Components disappeared, **PCTS:** Proximal Convoluted tubule shrunken, **DRC:** Degenerated renal corpuscle, **DE:** Detached epithelial cells from basal lamina.

4.5.2 Histopathological examination kidney on treatment with Heavy metalon day 10.

The 10milligrams per liter doses of heavy metals were exposed for 10 days, and a histopathological examination of the Kidney was observed. Obtained results explained that on exposure to 10 the Epithelial cell atrophy,detached epithelial cells from the basal lamina, Proximal convoluted tubule shrunken, tubular cell Components disappeared, Degenerated renal corpuscle, Basophilic cluster, Patchy distal tubule, Damaged podocyte cell were observed.

The 12milligrams per liter doses of heavy metals were exposed for 10 days, and a histopathological examination of the kidney was observed. Obtained results explained that on exposure to 12milligrams per liter the increased glomerulus space, Damaged glomerulus, Increased tubular lumen, Loss of first proximal tubule, Patchy degeneration, Separation of renal tubular epithelium from its basement, disorganized tubules, Visceral membrane damage, Red blood cells were observed.

The 24milligrams per liter doses of heavy metals were exposed for 10 days, and a histopathological examination of the kidney was observed. Obtained results explained that on exposure of 24 milligrams per literVacuolar degeneration, Damaged podocyte cells, Space of woman capsule, Detached epithelium from the basal lamina, Distal convoluted tube disappearance, Glomerular expansion, Dilation of Bowman's space, increasing in the diameter of renal tubules were observed.

The 26milligrams per liter doses of heavy metals were treated for 10 days, and a histopathological examination of kidney were observed. Obtained results, explained that on treatment with 26milligrams per liter dose detached epithelium cell from basel lamina, degenerated renal capsule, proximal conbulated tubule shrunken, tubular cell components disappeared, damaged podocyte cells were observed.

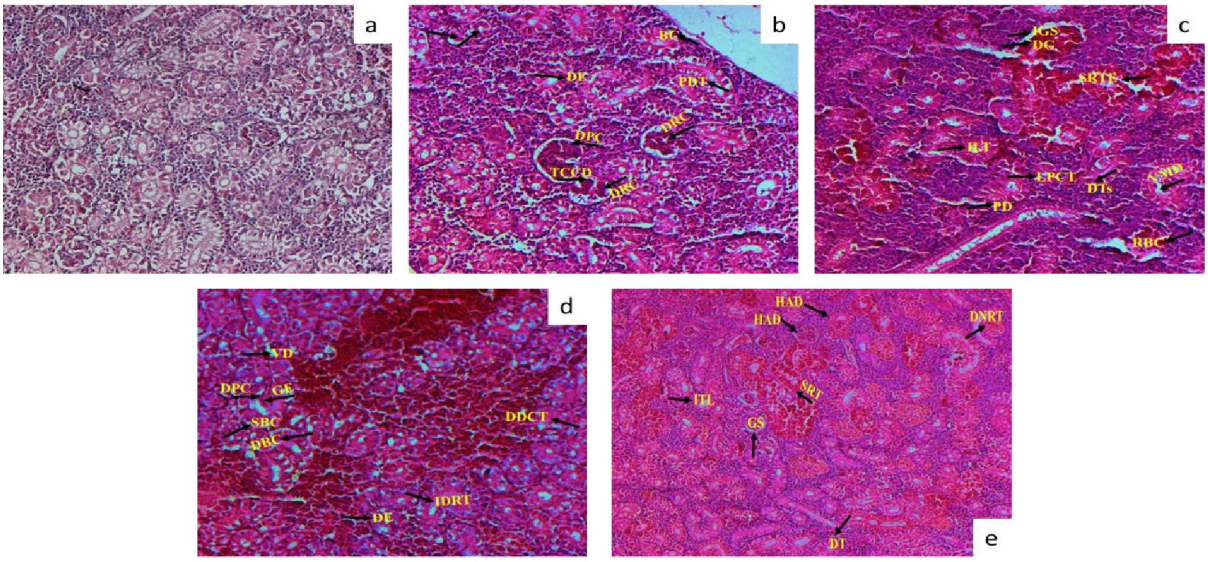

Figure 4.7 *C. punctatus* kidney section observations at 10 days, H&E at 100X. Control fish (a); Different concentrations of Heavy metal exposed fishes (b, 10milligrams per liter; c, 12 milligrams per liter; d, 24 milligrams per liter; e, 26 milligrams per liter).

DE: Detached epithelial cells from basal lamina, **AEC:** Atrophy epithelial cell, **SPCT:** shrunken Proximal convoluted tubule, **TCCD:** tubular cell, Components disappeared, **DRC:** Degenerated renal corpuscle, **BC:** Basophilic, Cluster, **PDT:** Patchy distal tubule, **DPC:** Damaged podocyte cell, **IGS:** increased glomerulus space, **DG:** Damaged glomerulus, **ITL:** Increased tubular lumen, **LPCT:** Loss of proximal Tubule, **PD:** Patchy degeneration, **SRTE:** Separation of renal tubular epithelium from its basement, **DTs:** Disorganized tubules, **VMD:** Visceral membrane damage, **RBC:** Red blood cells, **VD:** Vaculor degeneration, **DPC:** Damaged podocyte cells, **SBC:** Space of Bowman'S Capsule, **DE:** Detached epithelium form basal lamina **GE:** Glomerular Expansion, **DBC:** Dilation of Bowman's space, **IDRT:** increasing in the diameter of renal tubules, **DDCT:** disappeared Distal convoluted tube

4.6 Hematological observations:

Table 1 lists the values of the haematological parameters for each group of Heavy metal following exposure to fish at various doses. Red blood cells (RBCs), haemoglobin concentration, and haematocrit percentage (Hct) were significantly lower in the Heavy metal groups after both exposure times (p 0.05). For these factors, the exposure period's major effect was significantly

reduced (P 0.05). The (MCV) significantly decreased (p 0.05) as the dose of Heavy metal was increased. In all of the groups under observation, the mean corpuscular haemoglobin (MCH) did not demonstrate any appreciable variation. With increasing exposure to Heavy metal dosages, the mean corpuscular haemoglobin level (MCHC) showed a considerable rise.

Table 4.3 Summarization of Heavy metal different dose impact on Hematological parameters.

Concentration	10mg/ml		12 mg/ml		24 mg/ml		26 mg/ml		Control	
Blood Parameter	**5 D**	**10 D**	**5 D**	**10 D**	**5 Da**	**10 D**	**5 D**	**10 D**	**5 D**	**10 Days**
Hb (g/dl)	7.2±0.42	6.95±0.07	6.15±0.07	5±0.14	4.95±0.07	4.3±0.42	4±0	3.6±0.39	9.5±0.70	8.7±0.28
RBC ($x10^3/mm^3$)	6.1±0	5.1±0.28	5.0±0.0	4.10±0.0	5.23±1.05	5.05±0.07	4.85±0.07	4.78±0.02	6.25±1.20	6±1.55
MCV (fL)	20.45±0.62	20.54±0.74	21.80±0.84	19.6±0.59	24.04±1.20	21.55±0.72	21.05±0.07	20.43±0	23.0±9.87	20.34±5.29
MCH (pg)	13.35±0.36	11.77±0.67	11.35±0.07	10.40±0.41	13.39±0.70	11.67±0.15	12.1±0.94	10.32±0.45	11.60±0.67	11.85±0.04
MCHC (g/dL)	24.13±0.05	208.62±1.35	212.0±1.56	218.36±0.53	215±0.05	205.77±6.92	210.27±041	214.26±040	184.35±6.33	182.56±8.20
WBC ($x10^3/mm^3$)	23.0±1.23	1952.2±24.04	1302.5±60.10	1293.5±7.77	1914±1.41	1872.5±7.77	1805±9.89	126.5±0.70	4133±9.19	4277.8±69.29
Haematocrit (%)	15.77±0.84	15.47±1.13	12.1±0.28	10.4±028	15.70±071	15.28±0.54	15.11±0	14.48±0.22	20.84±0.67	21.26±0.41

4.7 Red Blood Cell (RBC)

The effect of similar exposure on RBC was decreased and findings are illustrated in the table. Data depicted that for 5 days of exposure with 10milligrams per liter, 12milligrams per liter, 24milligrams per liter, and 26milligrams per liter doses the RBC level were decreased and that were 6.1±0, 5.0±0.0, 5.23±1.05, 4.85±0.07 orderly. Similarly, for 10 days of exposure with 10milligrams per liter, 12milligrams per liter, 24milligrams per liter, and 26milligrams per liter doses the RBC level were 5.1±0.28, 4.10±0.0, 5.05±0.07, 4.78±0.02 respectively. Control value of RBC was6.25±1.20and 6±1.55 for 5^{th} and 10^{th} days respectively.

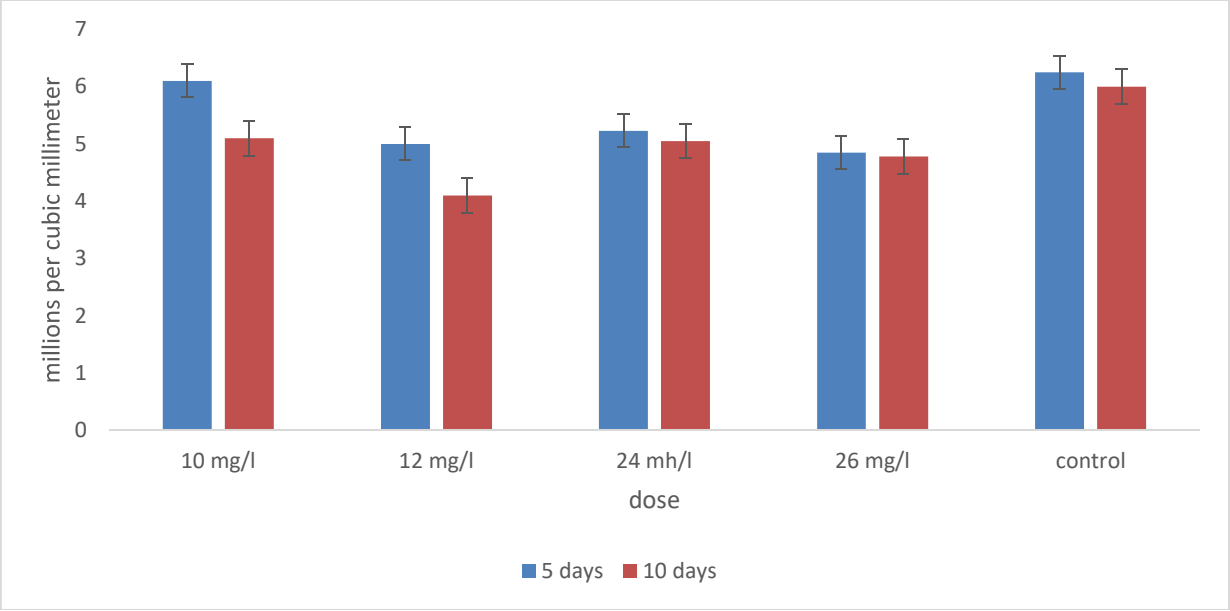

Figure 8: The RBC level in *Channa punctatus* were observed on exposing to different dose of heavy metal for 5 days and 10 days. The differences in the mean values among the treatment groups are statistically significant difference ($P < 0.05$).

4.8 Hemoglobin (Hb):

For 5 days of exposure with 10 milligrams per liter, 12 milligrams per liter, 24 milligrams per liter, and 26milligrams per liter doses the Hb level were decreased 7.2±0.42, 6.15±0.07, 4.95±0.07, and 4±0 respectively. In addition, for 10 days of exposure with 10milligrams per liter, 12milligrams per liter, 24milligrams per liter, and 26milligrams per liter doses the Hb level were 6.95±0.07, 5±0.14, 4.3±0.42, and 3.6±0.39 respectively. Control value of Hb was 9.5±0.70 and 8.7±0.28 for 5^{th} and 10^{th} days respectively. The effect of the exposure on hemoglobin level decreases as the concentration increases while comparing with control.

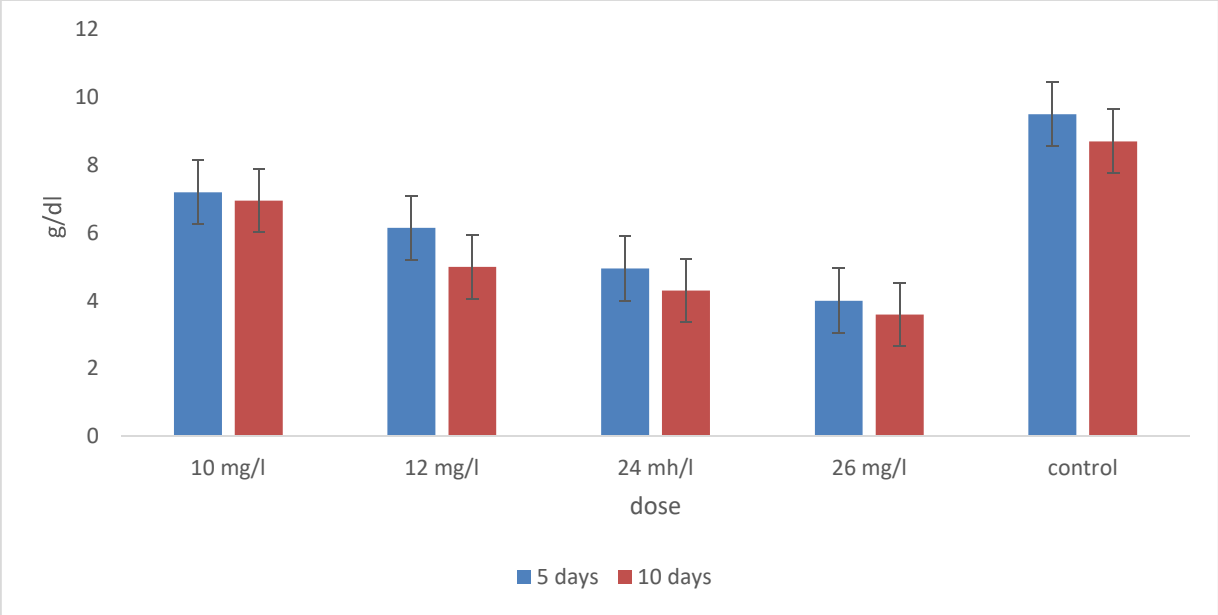

Figure 4. 9 The Hb level in *Channa punctatus* were observed at treating at different dose of heavy metal for 5 days and 10 days. The differences in the mean values among the treatment groups are less than would be expected by chance; there is a statistically significant difference ($P < 0.05$).

4.9 Hematocrit (Hct):

Finally, the exact doses were used during the investigation to assess the effect on the hematocrit levels. Addressing that, the Hematocrit levels were decreased with 15.77±0.84, 12.1±0.28, 15.70±071, and 15.11±0 for the 5 days of exposure with 10milligrams per liter, 12milligrams per liter, 24milligrams per liter, and 26milligrams per liter doses, respectively. While the MCH level was 15.47±1.13, 10.4±028, 15.28±0.54, and 14.48±0.22 following a 10-day exposure to 26 milligrams per liter, 24 milligrams per liter, 12 milligrams per liter, and 10 milligrams per liter doses, respectively. Control values of HCT were 20.84±0.67 and 21.26±0.41 for 5^{th} and 10^{th} days respectively.

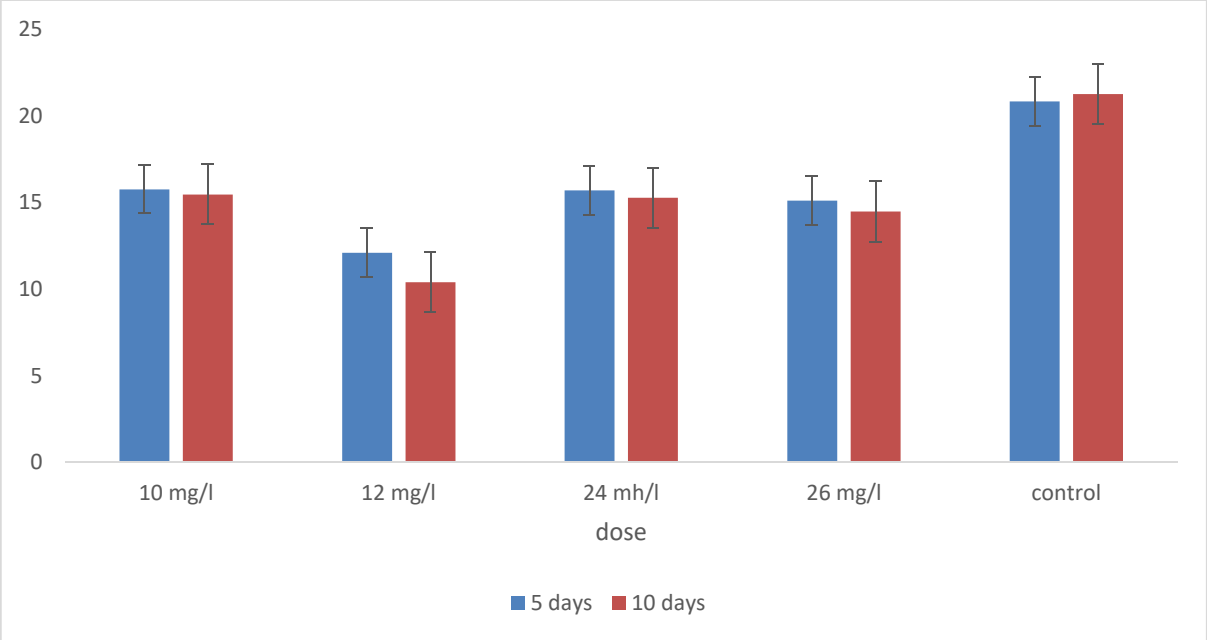

Figure 4.10 The Hct level (mean±SD) in *Channa punctatus* were observed at treating at different dose of heavy metal for 5 days and 10 days. The outcome is statistically significant difference ($P < 0.05$).

4.10 Mean cell volume (MCV):

Furthermore, the examination was carried out with the same exposure for getting the impact on MCV levels and found that the level decreases as compared to control of 5 days and 10 days. In respect to that for 5 days of exposure with 10milligrams per liter, 12milligrams per liter, 24milligrams per liter, and 26milligrams per liter doses the MCV level were decreased 20.45±0.62, 21.80±0.84, 24.04±1.20, 21.05±0.07 respectively. While for 10 days of exposure with 10milligrams per liter, 12milligrams per liter, 24milligrams per liter, and 26milligrams per liter doses the MCV level were 20.54±0.74, 19.6±0.59, 21.55±0.72, and 20.43±0 respectively. Control value of MCV were 23.0±9.87 and 20.34±5.29 for 5^{th} and 10^{th} days respectively

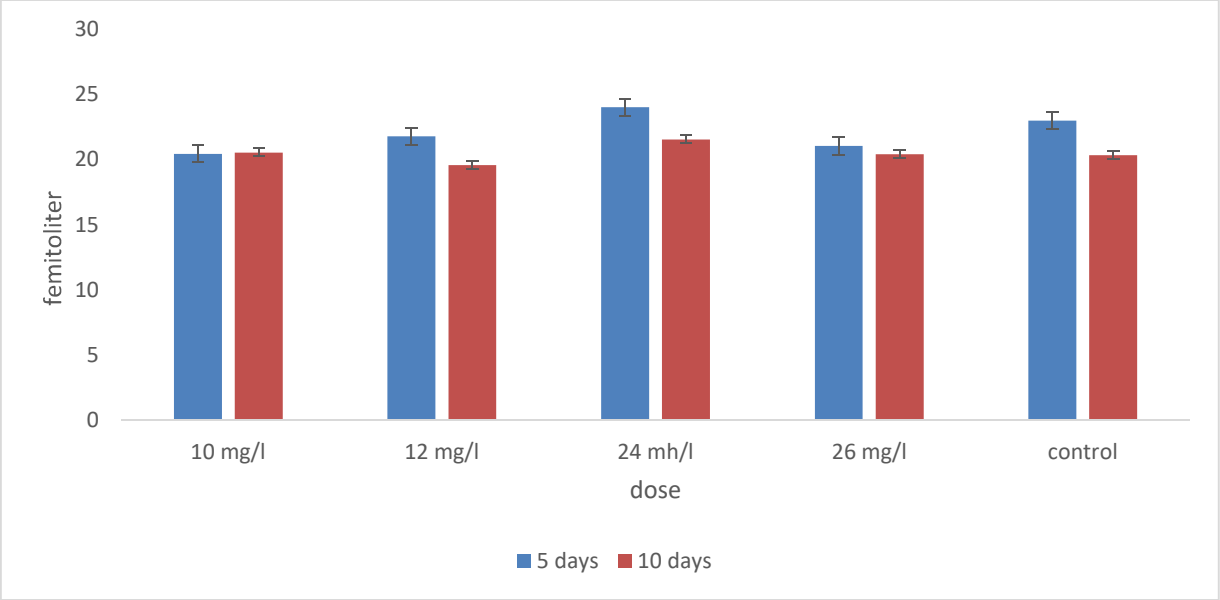

Figure 4.11 The MCV level (mean±SD) in *Channa punctatus* were observed on exposing to different dose of heavy metal for 5 days and 10 days. Values are expressed as (One Way ANOVA) the outcome is statistically significant i.e., ($p<0.05$).

4.11 Mean cell hemoglobin (MCH):

Additionally, the same exposure was used during the examination to determine the effect on MCH levels. Regarding that, the MCH level decreased 13.35±0.36, 11.35±0.07, 13.39±0.70, and 12.1±94 for the 5 days of exposure with 10milligrams per liter, 12milligrams per liter, 24milligrams per liter, and 26milligrams per liter doses, consecutively. While for 10 days of exposure, the MCH levels were 11.77±0.67, 10.40±0.4, 11.67±0.15, and 10.32±0.45 accordingly for doses of 26 milligrams per liter, 24 milligrams per liter, 12 milligrams per liter, and 10 milligrams per liter. The control value of MCH were 11.60±0.67and 11.85±0.04 for 5^{th} and 10^{th} days respectively.

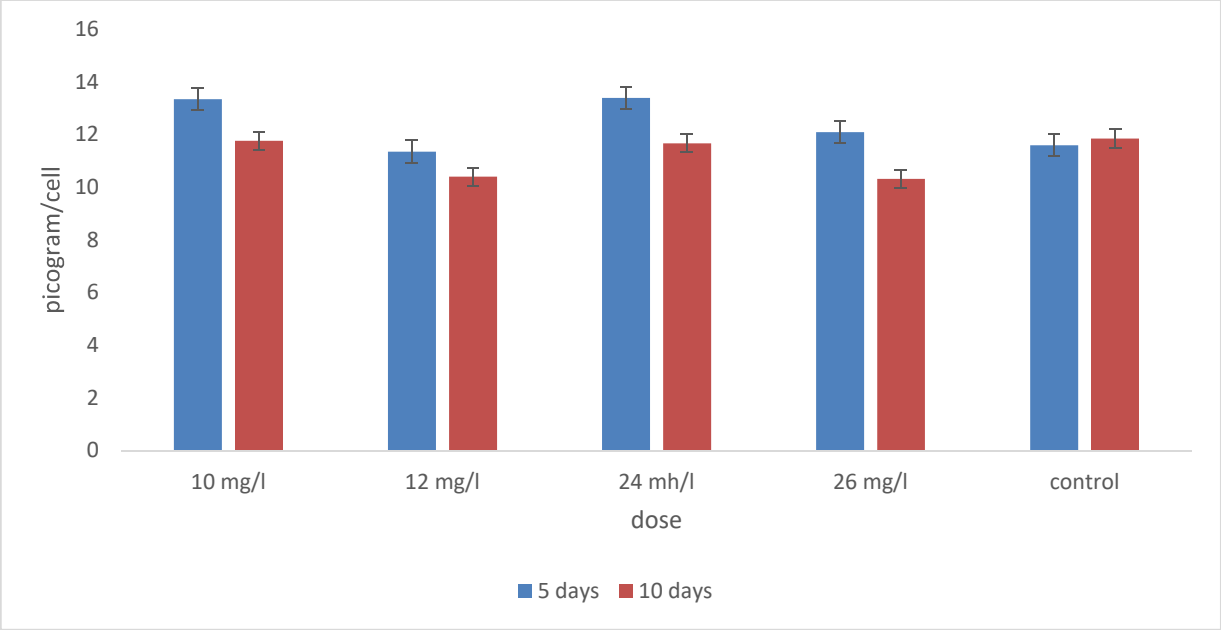

Figure 4.12 The MCH level (means) in *Channa punctatus* were observed at treating at different dose of heavy metal for 5 days and 10 days. Values are expressed as Mean±SEM (One Way ANOVA). The outcome is statistically significant difference ($P < 0.05$).

4.12 Mean cell hemoglobin concentration (MCHC):

Likewise, the very same treatment was used during the assessment to determine the effect on MCHC levels. Regarding that, the MCHC levels were increased with 24.13±0.05, 212.0±1.56, 215±0.05, and 210.27±041 for the 5 days of exposure with 10milligrams per liter, 12milligrams per liter, 24milligrams per liter, and 26milligrams per liter doses, correspondingly. Although for 10 days of exposure, the MCHC levels were 208.62 ±1.35, 218.36 ±0.53, 205.77 ±6.92, and 214.26± 040, respectfully. Control value of MCHC were 184.35±6.33 and 182.56±8.20for 5^{th} and 10^{th} days respectively

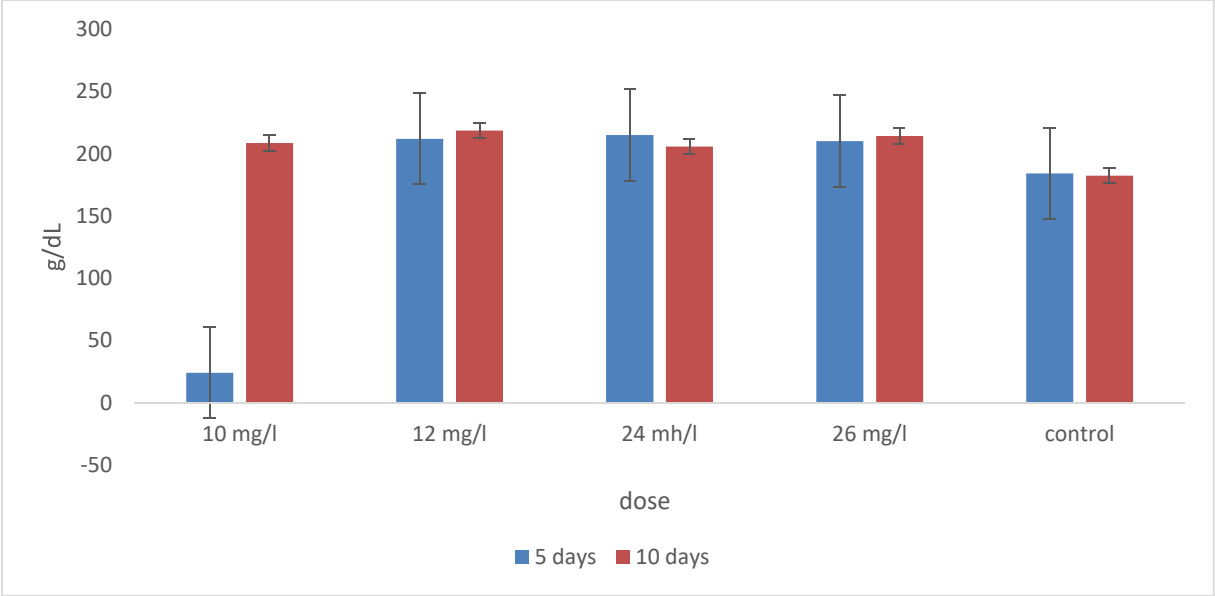

Figure 4.13 The MCHC level (mean±SD) in *Channa punctatus* were observed on exposing to different dose of heavy metal for 5 days and 10 days the outcome is statistically significant i.e., ($p<0.05$).

4.13 White Blood Cells (WBC):

Moreover, the identical dosage was employed during the investigation to determine the influence on WBC levels. In connection to that, the WBC levels were increased with 23.0± 1.23, 1302.5 ±60.10, 1914 ±1.41, and 1805 ±9.89 for the 5 days of exposure with 26 milligrams per liter, 24 milligrams per liter, 12 milligrams per liter, and 10 milligrams per liter doses, consecutively. Whereas for 10 days of exposure, the WBC levels were 1952.2 ±24.04, 1293.5± 7.77, 1872.5 ±7.77, and 126.5 ±0.70, accordingly. Control value of WBC were 4133±9.19 and 4277.8±69.29 for 5^{th} and 10^{th} days respectively

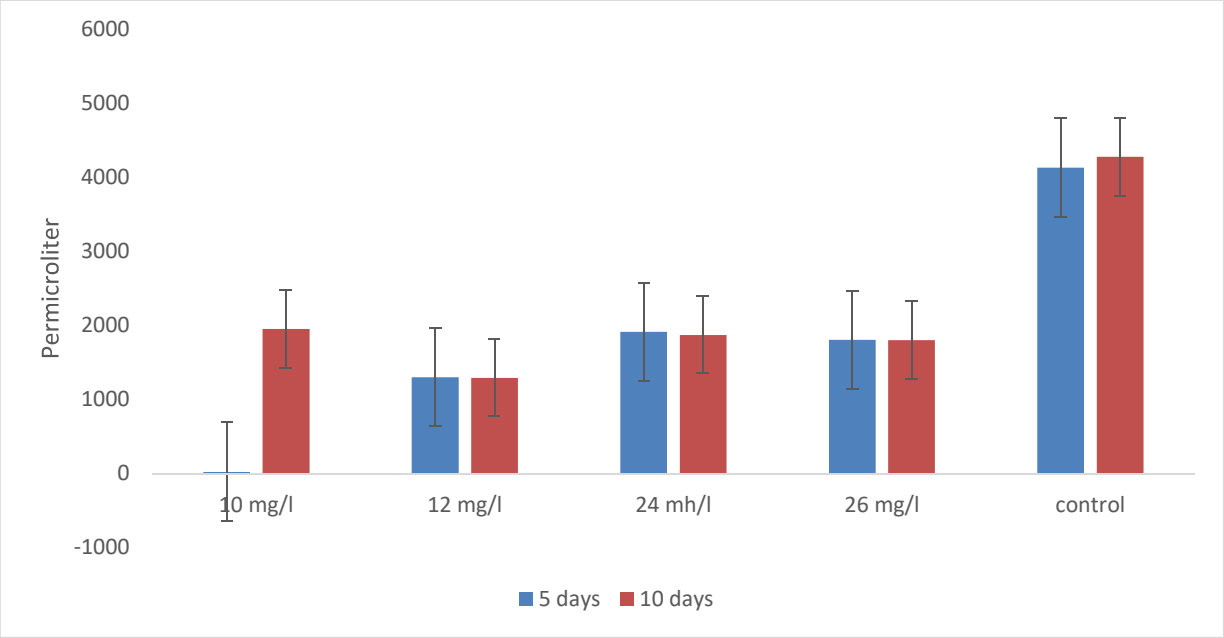

Figure 4.14 The WBC level (mean±SD) in *Channa punctatus* were observed on exposing to different dose of heavy metal for 5 days and 10 days. Statically significant difference comparatively to control if ($p<0.05$).

Superoxide dismutase activity

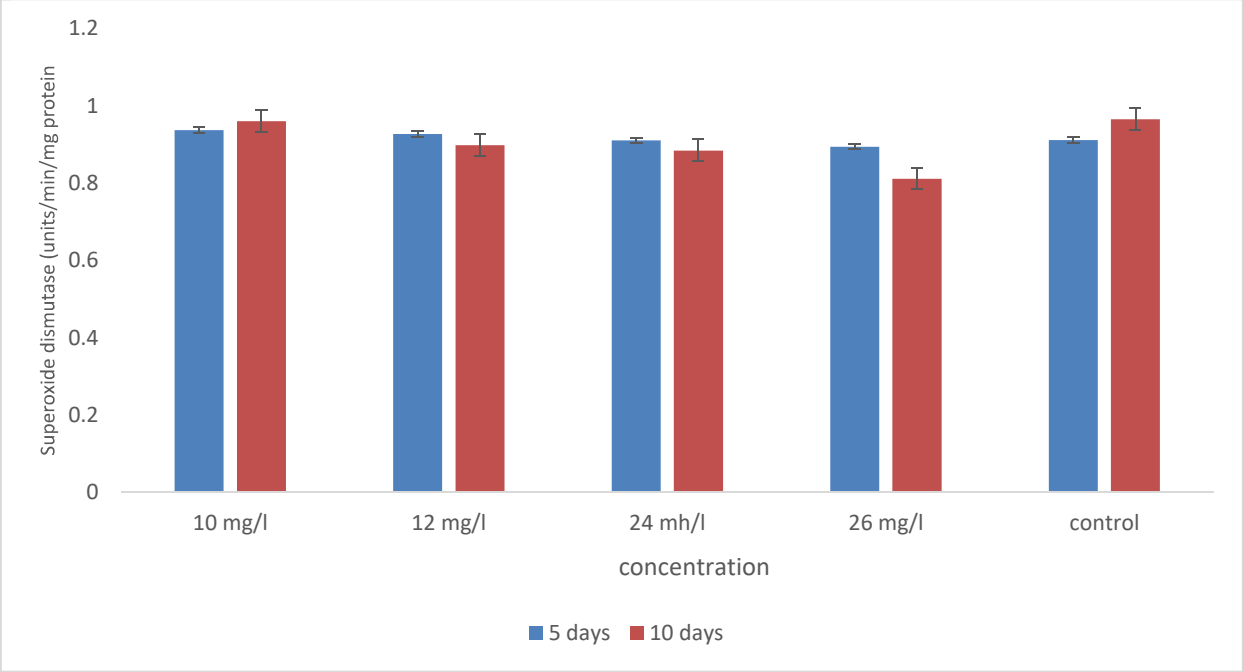

Figure 4.15 Illustration of research findings showing the impact of various heavy metal concentrations on fish exposure and the resulting changes in super oxide dismutase function in their gills.

Catalase (units/min/mg protein)

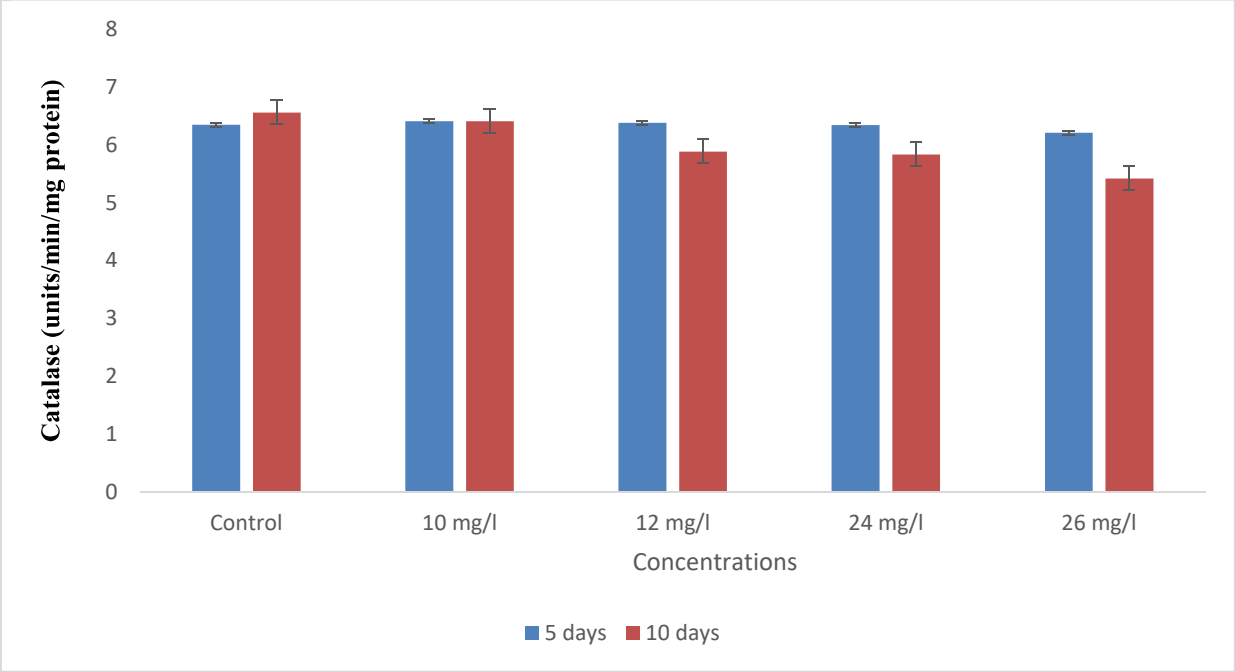

Figure 4.16 Illustration of research findings showing the impact of various heavy metal concentrations on fish exposed to them and how it affected their gills' catalase activity.

Lipid peroxidation (µmoles/MDA/mg protein)

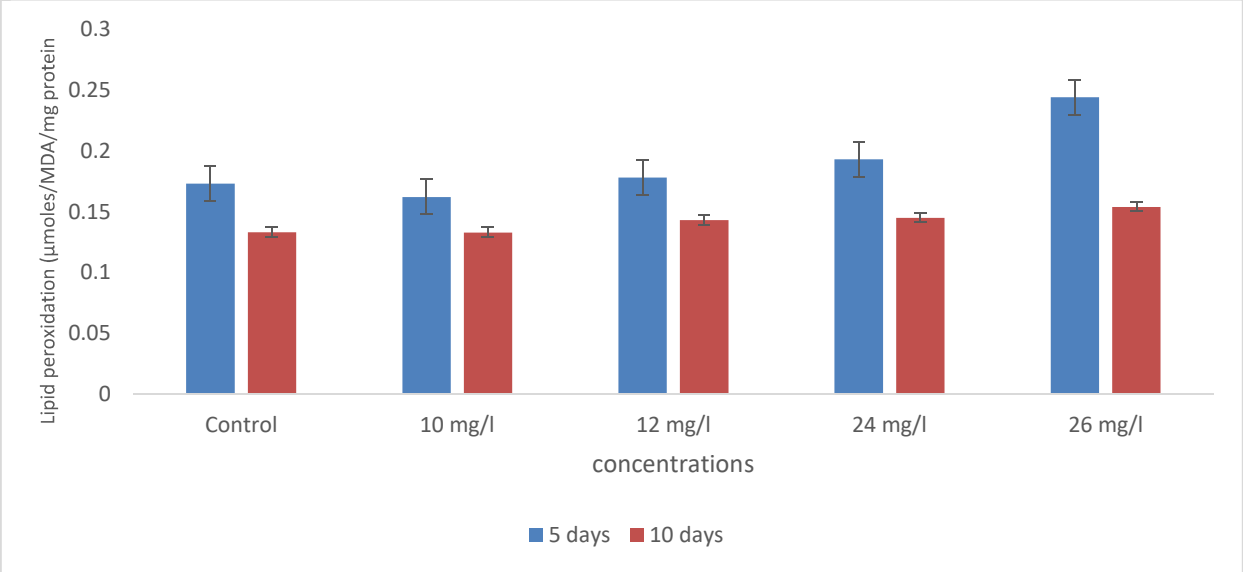

Figure 4.17 An illustration of research findings showing the impact of varying heavy metal concentrations on fish exposure and the gills' lipid peroxidation activity

.

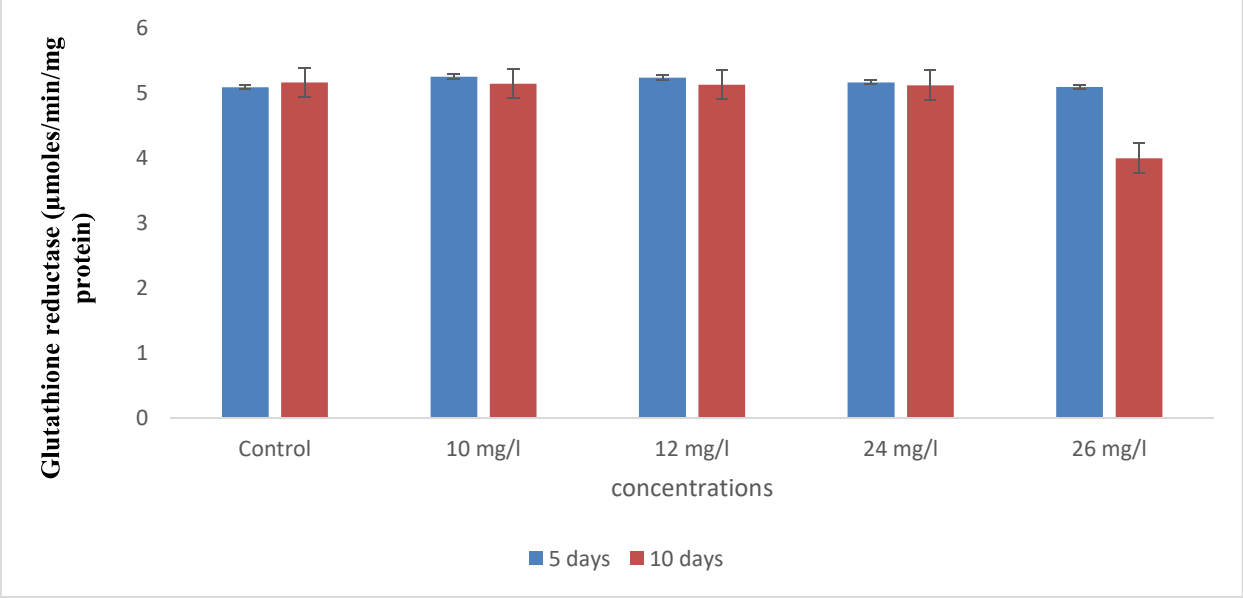

Figure 4.18 Illustration of research findings showing the impact of varying heavy metal concentrations on fish exposure and the activity of glutathione reductase in the gills.

Glutathione-s-transferase (µmoles/min/mg protein)

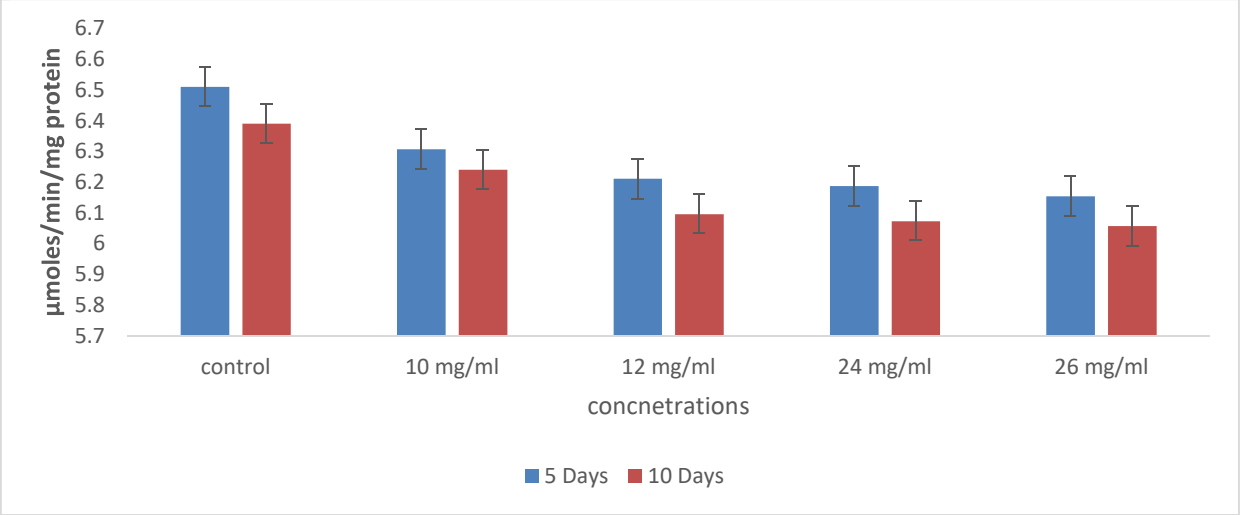

Figure 4.19 Illustration of research findings showing the impact of varying heavy metal concentrations on fish exposure and how it affected the activity of glutathione-s-transferase (µmoles/min/mg protein) in the gills.

Glutathione peroxidase (µmoles/min/mg protein)

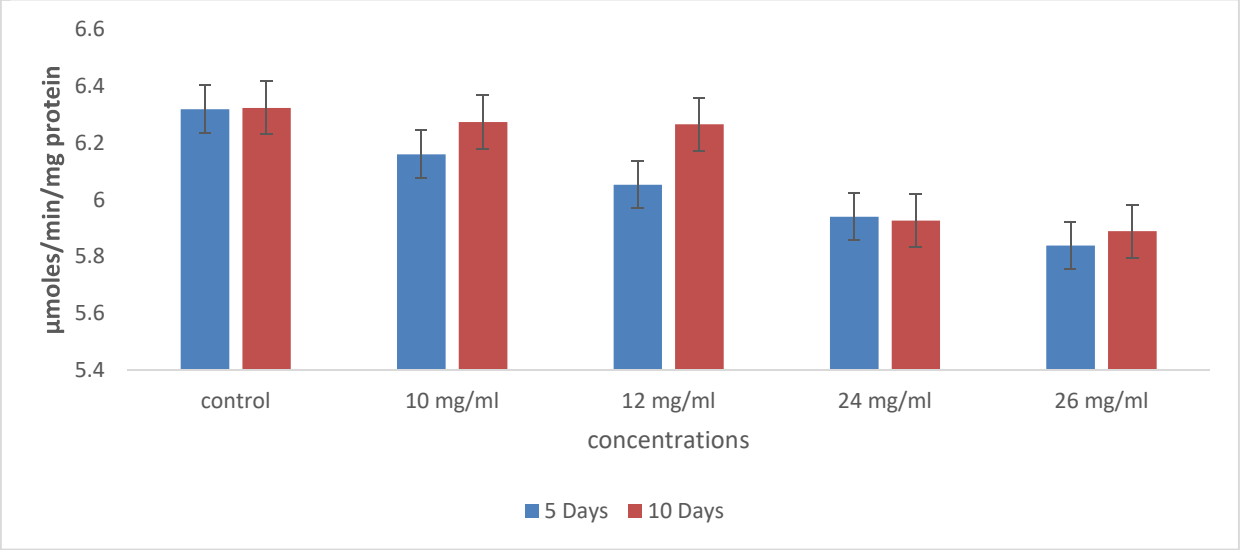

Figure 4.20 Visual depiction of findings from studies wherein fish exposed to varying concentrations of heavy metals and their impact on glutathione peroxidase (µmoles/min/mg protein) activity in their gills was studied.

Kidney

Superoxide dismutase (units/min/mg protein)

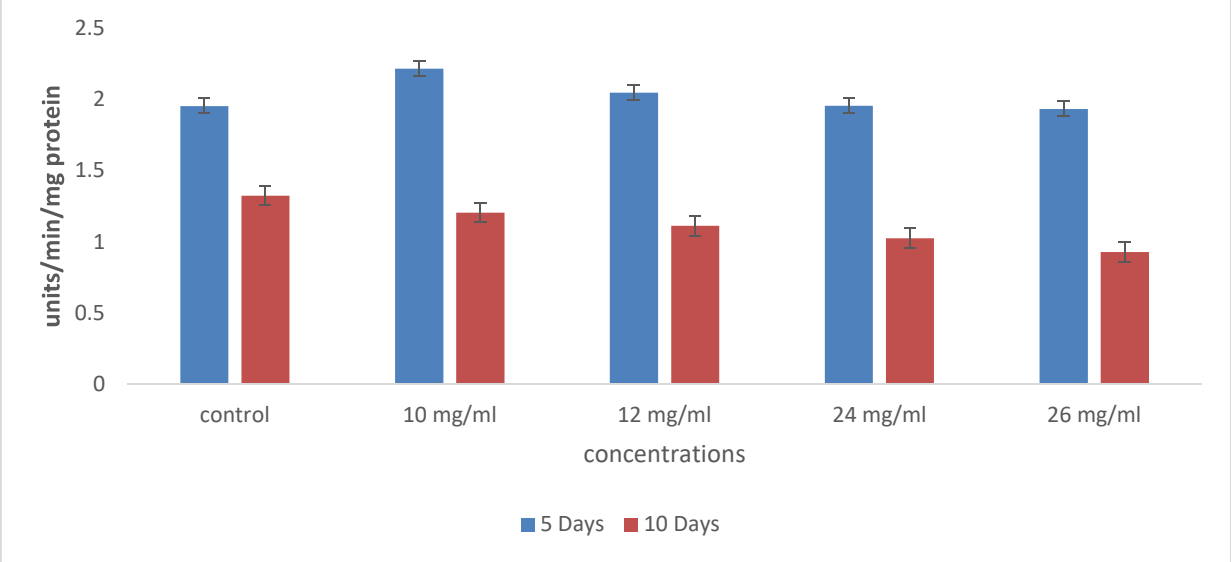

Figure 4.21 Illustration of research findings showing the impact of various heavy metal concentrations on fish exposure and the activity of superoxide dismutase in their gills.

Catalase (units/min/mg protein)

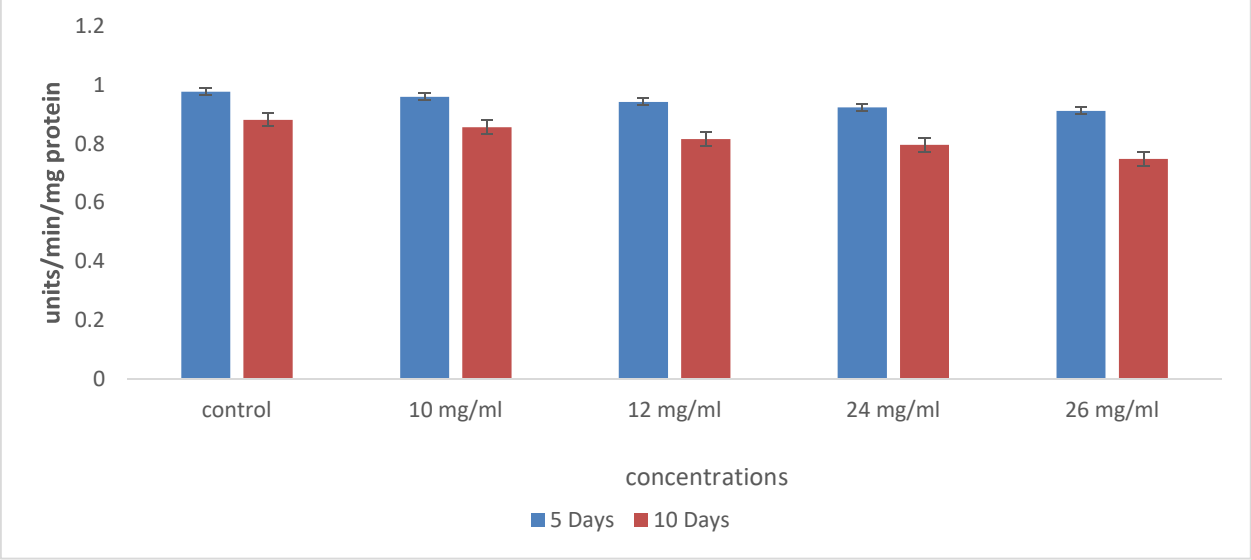

Figure 4.22 A visual depiction of the findings from a study that examined the effects of various heavy metal concentrations on fish exposure and kidney catalase function.

Lipid peroxidation (µmoles/MDA/mg protein)

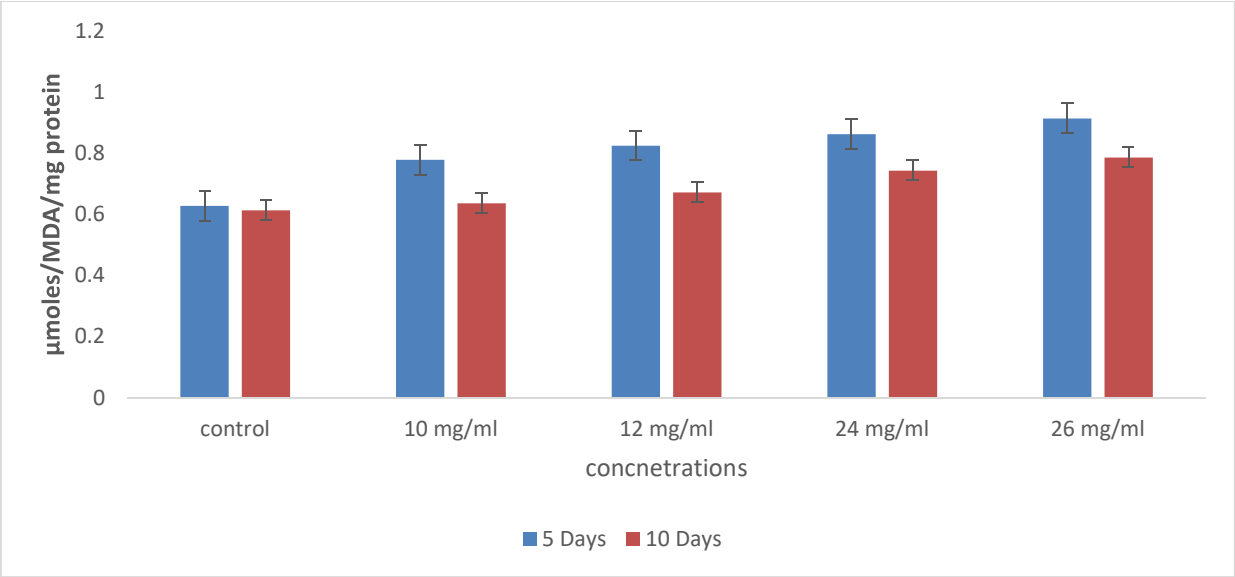

Figure 4.23 An illustration of the results of a study that examined the effects of various heavy metal concentrations on fish exposure and the kidney's lipid peroxidation activity

Glutathione reductase (µmoles/min/mg protein)

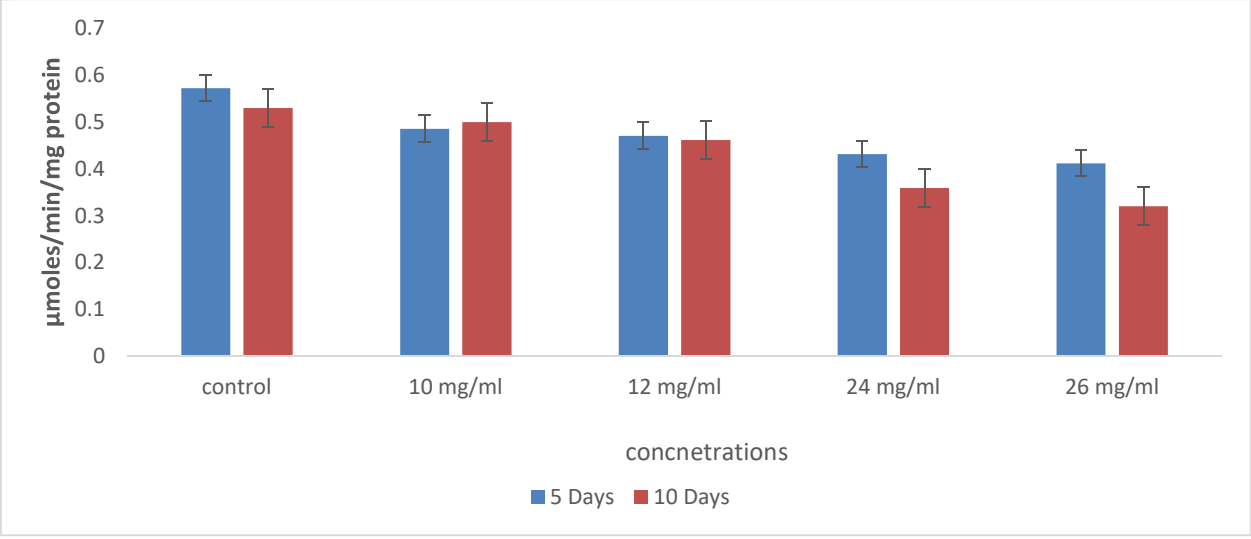

Figure 4.24 An illustration of the results of a study that examined the effects of various heavy metal concentrations on fish exposure and the kidney's lipid peroxidation activity

Glutathione-s-transferase (µmoles/min/mg protein)

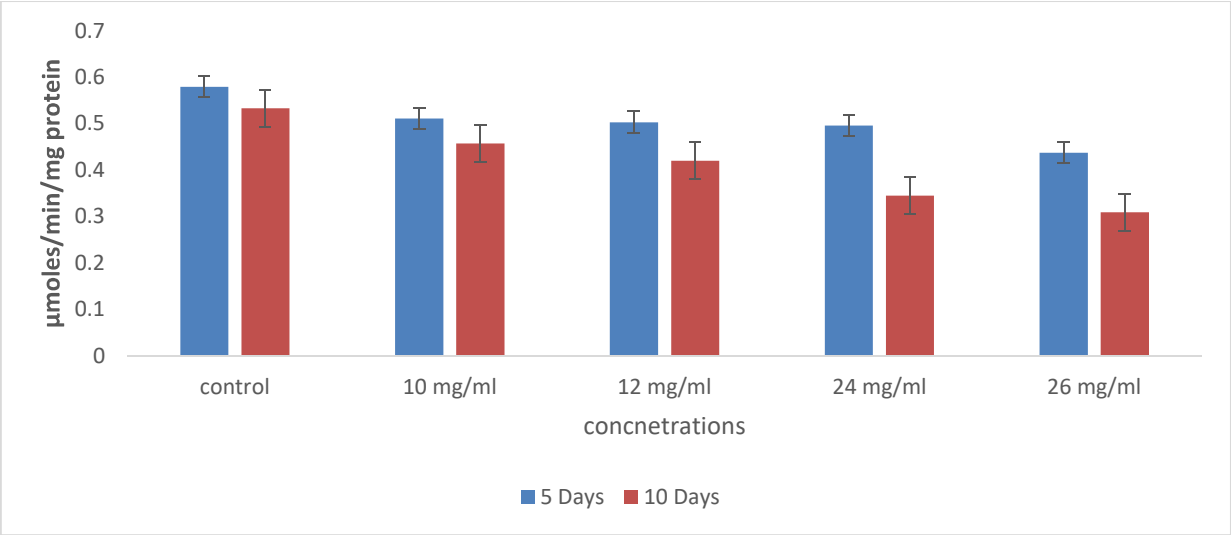

Figure 4.25 A visual depiction of the findings from a study that examined the effects of varying heavy metal concentrations on fish exposure and the kidney's Glutathione-s-transferase activity (µmoles/min/mg protein).

Glutathione peroxidase (µmoles/min/mg protein)

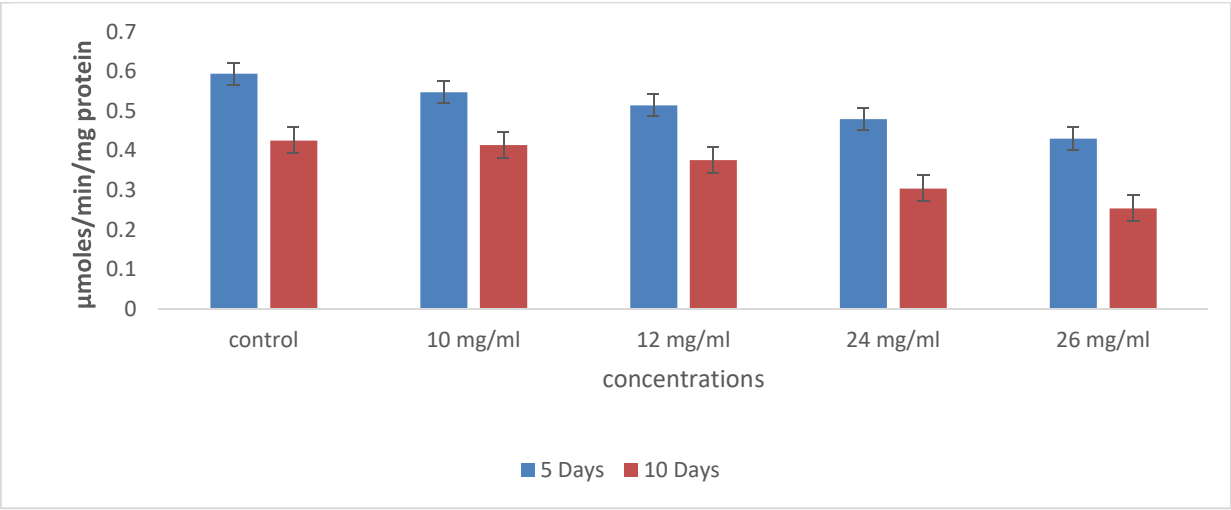

Figure 4.26 Illustration of research findings showing the impact of various heavy metal concentrations on fish exposure and the resulting changes in glutathione peroxidase (µmoles/min/mg protein) activity in the kidney.

Muscles:

Superoxide dismutase (units/min/mg protein)

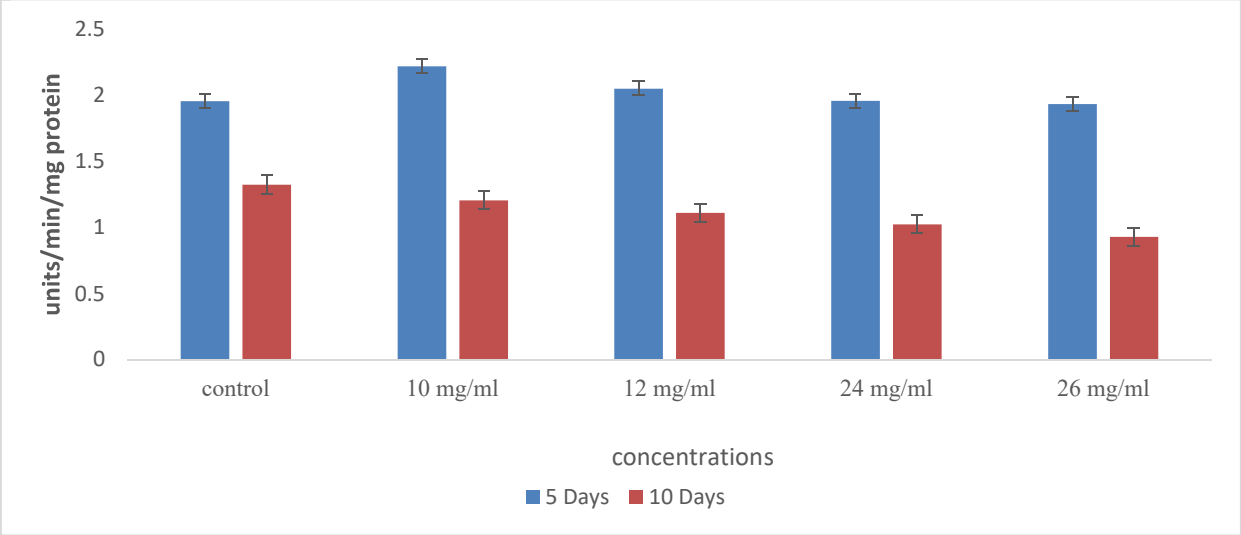

Figure 4.27 Illustration of research findings showing the impact of various heavy metal concentrations on fish exposure and the resulting changes in superoxide dismutase activity in the muscles.

Catalase (units/min/mg protein)

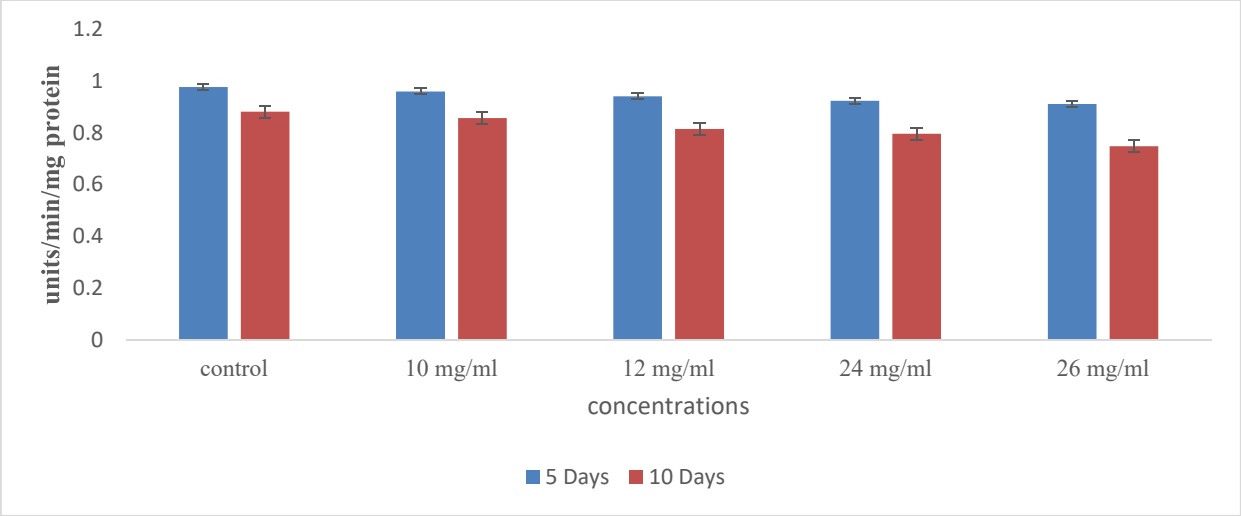

Figure 4.28 Illustrative depiction of findings derived from a study examining the impact of varying levels of heavy metal exposure on fish, specifically focusing on the activity of Catalase (units/min/mg protein) in their muscles.

Lipid peroxidation (µmoles/MDA/mg protein)

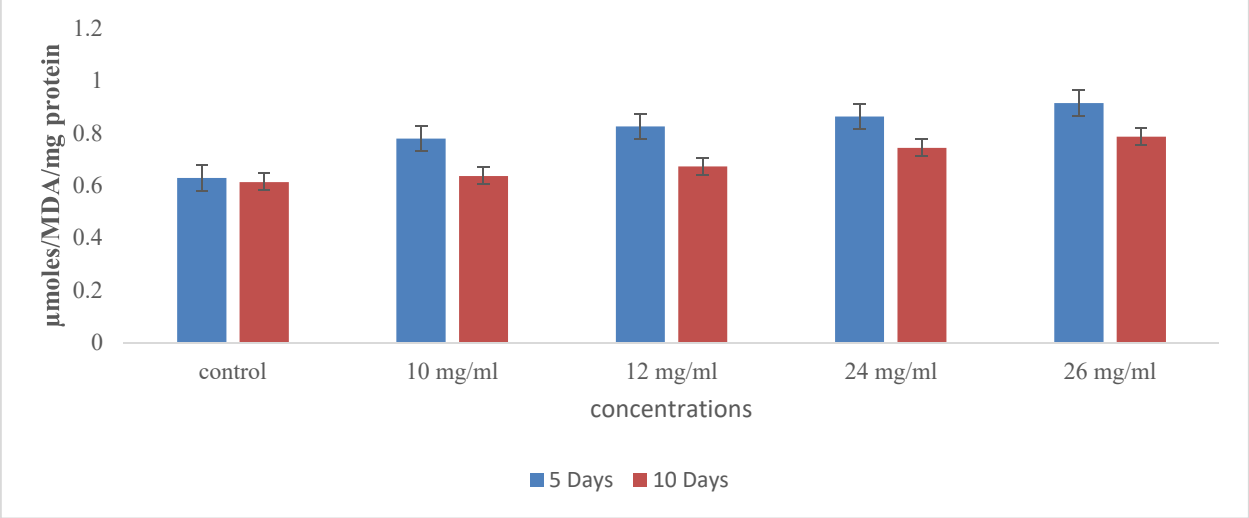

Figure 4.29 Examining the impact of varying concentrations of heavy metals on fish, researchers observed the resulting effects on lipid peroxidase activity in muscle tissue. The findings were visually represented in a pictorial format.

Glutathione reductase (µmoles/min/mg protein)

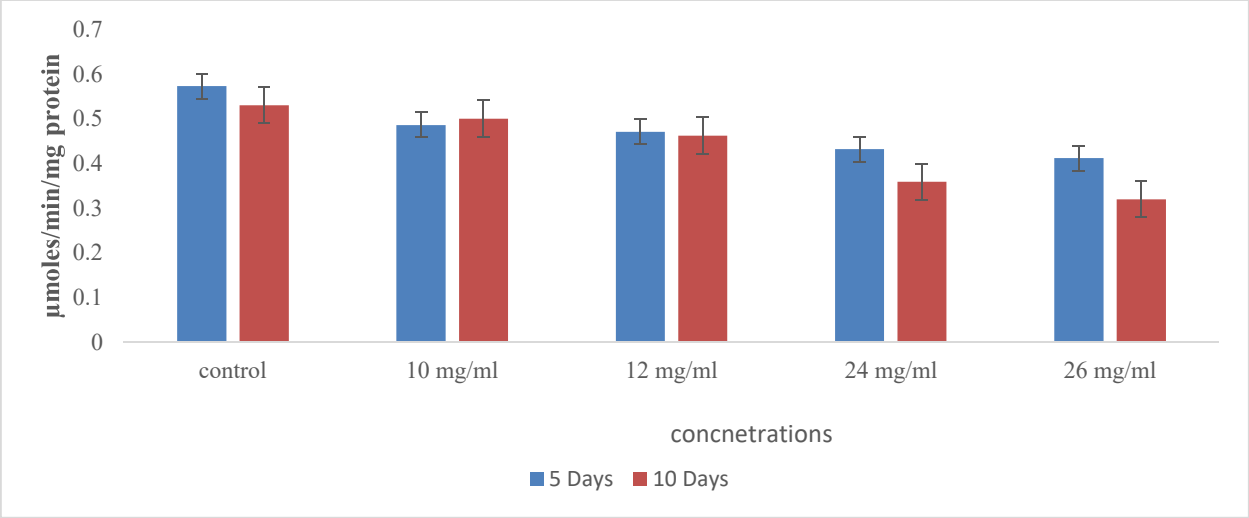

Figure 4.30 A graphic was presented showcasing the results of a research study that examined the impact of different concentrations of heavy metal on fish. Specifically, the study focused on how these concentrations affected the activity of glutathione reductase in the muscles of the fish, measured in µmoles/min/mg protein.

Glutathione-s-transferase (µmoles/min/mg protein)

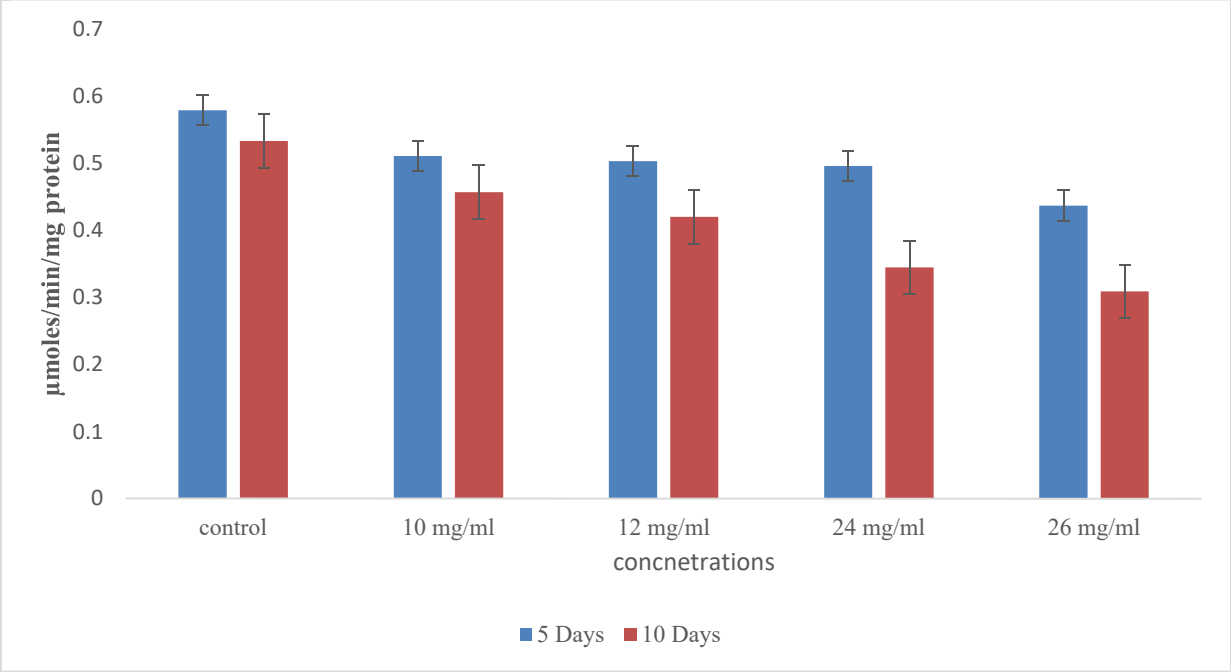

Figure 4.31 Observations were made on the impact of varying concentrations of Heavy metal on fish, specifically focusing on the activity of glutathione s-transferase in their muscles.

Based on the findings of this experiment, it was observed that the activity of glutathione s-transferase (moles/min/mg protein) decreased significantly as the concentration of heavy metals and exposure time increased ($P < 0.05$). In addition, after incubating for 5 days at concentrations of 10 ppm, 12 ppm, 24 ppm, and 26 ppm, the activities of Glutathione s-transferase were measured at 0.511 µmoles/min/mg proteins. The measured values for the activity of the enzyme were 0.503 µmoles/min/mg protein, 0.496 µmoles/min/mg proteins, and 0.437 µmoles/min/mg proteins, respectively. In comparison, the control group had an activity of 0.579 µmoles/min/mg protein. For a 10-day incubation period at concentrations of 10 ppm, 12 ppm, 24 ppm, and 26 ppm, the catalase activities were measured at 0.457 µmoles/min/mg protein. The enzyme activity levels in the experimental group were 0.42 µmoles/min/mg protein, 0.345 µmoles/min/mg proteins, and 0.309 µmoles/min/mg proteins, respectively. In comparison, the control group had a glutathione s-transferase activity of 0.533 µmoles/min/mg protein.

Glutathione peroxidase (µmoles/min/mg protein)

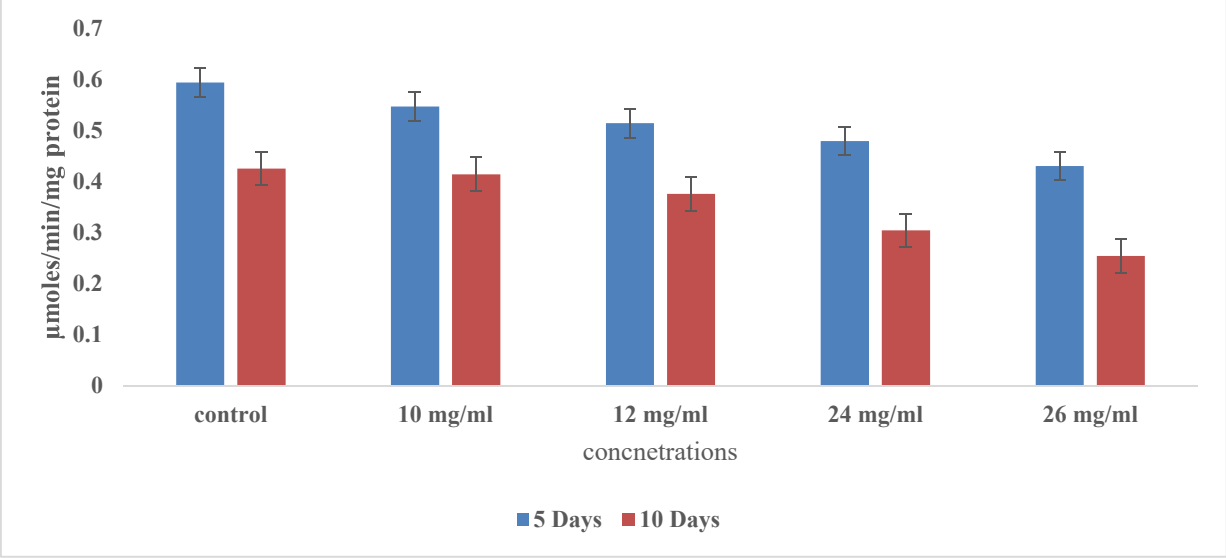

Figure 4.32 An analysis was conducted on the impact of varying levels of Heavy metal on fish, specifically focusing on the activity of glutathione peroxidase in their muscles. The results of these studies were visually represented to highlight the findings.

Gene expression

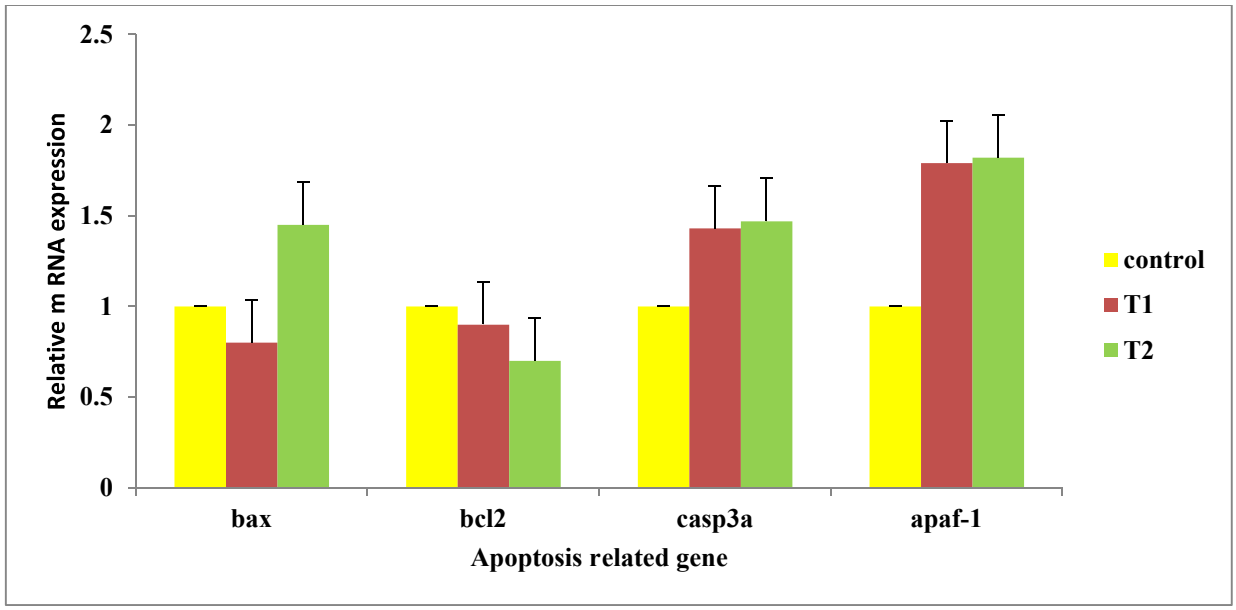

Figure 4.33 The impact of Pb^{2+} stimulation on the mRNA levels of genes associated with apoptosis is illustrated in Figure 1.

The quantity of Pb^{2+} and the duration of treatment have a notable interactive influence on the activity of genes related to apoptosis ($P < 0.05$). After being exposed to Pb^{2+} for 5 days, the levels of bax as well as bcl2 decreased dramatically, while the levels of casp3a and apaf-1 in the liver were substantially greater in the group treated with 12 ppm compared to the control group ($P < 0.05$). After 10days of treatment with $Pb^{2+,}$ the level of expression of bax, casp3a, and apaf-1 was found to be raised, whereas the transcription of bcl-2 was found to decrease in comparison to the group acting as a control.

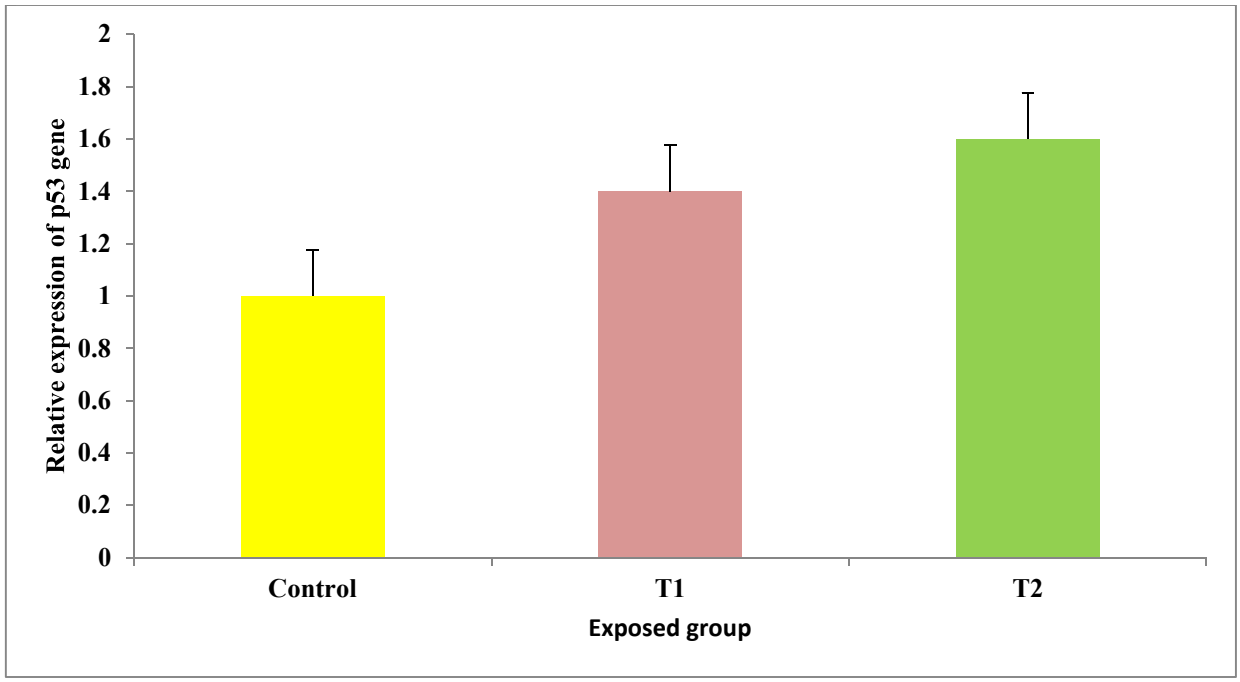

Figure 4.34 The impact of Pb2+ treatment on the mRNA levels of genes associated with apoptosis is illustrated in Figure 2.

The quantity of Pb2+ and the duration of exposure have a notable interactive influence on the transcription of genes related to apoptosis ($P < 0.05$). On the 30th day of treatment to Pb2+, there was a substantial increase in the expression of the p53 gene in comparison to the members of the control group.

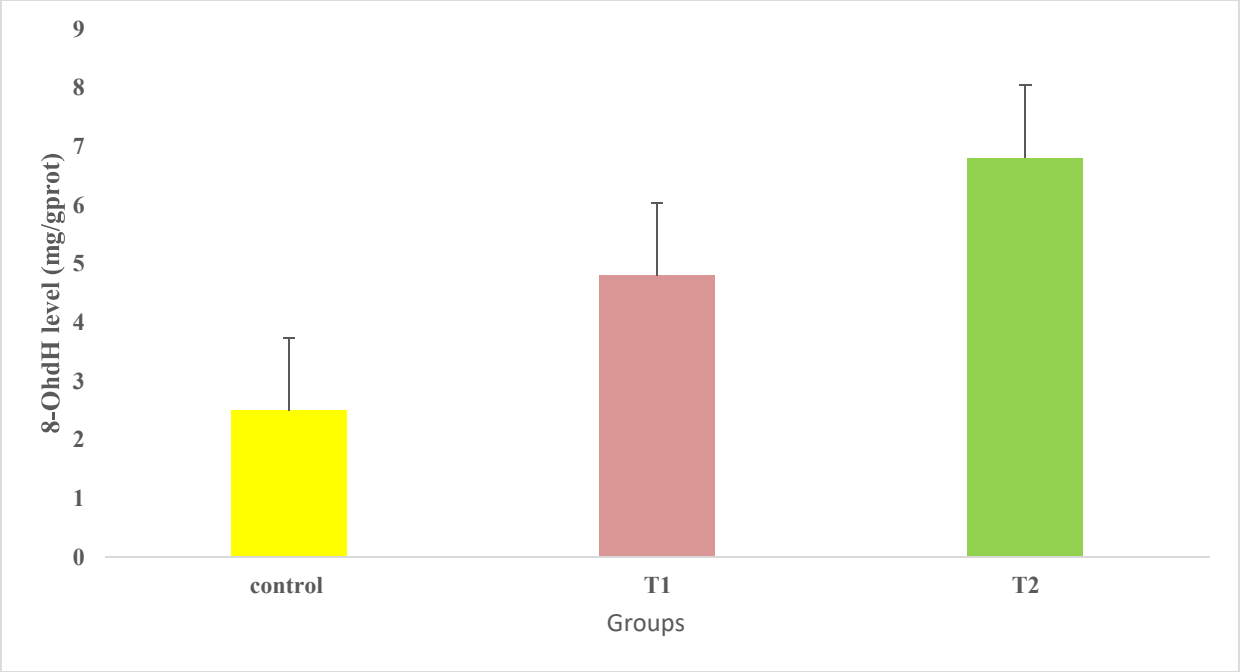

Figure 4.35 Changes in 8-OHdG levels were observed following exposure to different concentrations of Pb2+ for periods of 5 days and 10 days.

The values are displayed as the average ± the standard deviation. Differences between groups that are considered significant ($P < 0.05$) 8-OHdG are a shortened form of the chemical compound known as 8-hydroxy-2 deoxyguanosine.

Chapter-5

DISCUSSION

Fish in the study became lethargic and displayed obvious parasite illnesses along with pale body colors after five and ten days of exposure to all Lead (Pb) concentrations. The fish's pale color was due to anemia, which was brought on by the harmful effects of lead (Pb), according to Gomez *et al.,* (2021) analysis of the fish's hematology. Furthermore, using lead (Pb) at any level caused oxidative stress, which diminished the activity of cell-protective enzymes, raised LPO levels, and caused serious tissue disorders in *Channa fish.*

The organ most affected by lead (Pb) exposure was the liver of the fish. Consequently, the release of lead (Pb) into the aquatic ecosystem may pose a threat to aquatic life. Thus, aquatic life may be at risk from Lead (Pb) discharged into water bodies. It is difficult to predict the behavior, destiny, and toxicity of lead (Pb) after it is released into the environment. Lead (Pb) is categorized as a Group B carcinogen by the IARC, most likely due to its ability to cause cancer in humans (Steenland, K., & Boffetta, P. (2000).

Lead (Pb) concentrations in aquatic habitats vary from 0.7 to 16.8 micrograms per litre. Furthermore, it is estimated that 15600 mg of lead (Pb) enter aquatic habitats globally annually. Understanding how Lead (Pb) affects aquatic animals is essential. As can be observed, the majority of these studies found no acute effects when Lead (Pb) concentrations were greater than 30 mg/lit, which is in line with our results. Because lead (Pb)'s size and other parameters affect its properties, destiny, and behavior in biological systems, characterizing lead (Pb) is crucial for eco-toxicity research (Kumar, *et al.,* 2020). In rainbow trout, intraperitoneal injection of lead (Pb) resulted in kidney bioaccumulation and almost no removal even ninety days later (Rogers, J. T. 2003). According to a different recent study, rainbow trout kidneys could accumulate up to 94% of lead (Pb) after receiving an intravenous injection (101).

Oxidative:

Lead (Pb) incorporation, a key mechanism of toxicity and one of the causes of Lead (Pb) poisoning, results in ROS formation after phagocytosis (Metryka *et al.,* 2018). Similar to humans, fish possess highly developed antioxidant defense mechanisms that help them

resist the harmful effects of lead. The potential for UV absorption and photocatalytic activity increases with lead (Pb) particles. Lead (Pb) may readily pass through cell membranes, thereafter it interacts with intracellular metabolism to form ROS and pro-oxidant effects in the cells with which it comes into contact with Behra and Krug. Certain findings on in-vitro and in-vivo investigations revealed changes in oxidative stress indicators in fish exposed to lead (Pb). Lead (Pb) can create reactive oxygen species (ROS), particularly the OH (Behra, R., & Krug, H. 2008).

These defense mechanisms comprise low-molecular-weight, non-enzymatic antioxidants like GSH as well as enzymes like CAT, and SOD. The primary line of defense against oxidative harm at the cellular level is the CAT, POD, and SOD system. The three Lead (Pb) 4 doses administered to the Channa in this investigation ranged from 30 milligrams to 90 milligrams each litter. It was clear that these exposures altered the antioxidant enzyme function in numerous organs (Sharma, *et al.,* 2019). When rainbow trout were subjected to Lead (Pb) at levels below the fatal threshold, oxidative damage and a rise in peroxidation of lipids were noted, likewise, oxidative stress was caused by oxygen free radicals generated in the gill, and liver, along with gut tissues of zebra fish subjected to $CuSO_4$,ZnO_4. Additional heavy metals Similarly, antioxidant enzyme activity was reduced in the gill as well as liver tissues of *Oreochromis mossambicus* fish exposed to $CdCl_2$ at dosages of 25, 50, and 75 milligrams per liter for a total of eight days (Mohammed Abdulridha, J. A. 2016).

Fish exposed to lead (Pb) may also lose their hematopoietic cells as well as immune system in both their posterior and anterior kidneys, which could lead to a weakened antimicrobial response. The loss of hematopoietic and immune cells in the fish kidney following the ingestion of heavy metals appeared to be an established histopathology characteristic, regardless of the type of NO. Lead (Pb) toxicity to juvenile carp has been documented. This toxicity may be caused by a ROS-induced poisoning pathway, whereby CuSO4 causes oxidative strain, which leads to LPO, and subsequently impacts cellular metabolic defense activities (Zou *et al.,* 2016). Prior research revealed that exposure to C60 resulted in LPO in the brains of largemouth bass. Increased levels of LPO were seen in the liver and gill tissues of rainbow trout who received SWCNT, according to results on oxidative damage. When subjected to Lead (Pb) at levels of 5 and 10 milligrams per liter,

aquatic invertebrates such as Daphnia magna demonstrated notable alterations in antimicrobial enzymes such as SOD,CAT, GPX, and GST in according to concentration. Lead (Pb) enhanced the rates of lipid oxidation and changed the activities of antioxidant enzymes (CAT, SOD, and POD) in *Cyprinus carpio* (Raza *et al*., 2016).

SOD: The reduction in SOD activity to remove antioxidants from the muscular tissues of C. gariepinus. The activity of SOD in the carp's brain and gill had dramatically dropped, according to Linhua *et al.,* (2009). Li *et al*., (2023) reported a decrease in SOD levels in tissues of the liver following exposure to lead (Pb). Consistent with this, adult Japanese medaka livers demonstrated a significant decrease in SOD activity following exposure to nano iron. However, there was a brief increase in SOD activity at 40 and 90 milligrams per liter $CuSO_4$, which was followed by a sharp fall, indicating that oxidative stress was caused by increased ROS generation and compromised defenses.

According to Chen *et al.,* (2012), Lead (Pb) had a negative impact on Danio rerio at concentrations between 1.0 and 7.0 milligrams per liter, which decreased the rerio's liver weight ratio. Lead (Pb) treatment can change several organs' molecular, physiological, as well as histological makeup. Although the manufacturer accurately describes the physicochemical characteristics of Lead (Pb) tested in powder form, the material acts differently in suspension by aggregating, forming clusters, and regimenting. We used Lead (Pb) tracking analysis electron microscopy to examine samples of Lead (Pb) in aqueous solutions. Despite having a primary size of 50 nm, the results indicated the suspension of a variety of Lead (Pb) particle sizes, with a heterogeneous size distribution ranging from nanoscale to microscale.

The efforts to explain how given Lead (Pb) interact with biological structures and how they are administered are challenging due to the availability of a range of heavy metals features mimicking the behavior of test materials in biological contexts. As a result, it is challenging to carry out repeatable and trustworthy toxicological investigations with heavy metals. It was discovered that the tested doses were not fatal for the examined fish for the course of the exposure to Lead (Pb) (10 days). This can partly be explained by the fact that *C. auratus* is known to be resistant to environmental changes and contaminants created by humans, such as heavy metals or organochlorine insecticides (Holmstrup, *et al.,* 2010)

Even said, the fact that *C. puntatus* appears to have little acute toxicity does not imply that there are no harmful consequences. In reality, as our findings indicate, the organism's organs exhibit a number of histological and biochemical alterations. After exposing rainbow trout to Lead (Pb) for 14 days, Boorges *et al.,* (2013) discovered findings that were comparable. There is evidence that exposure to Lead (Pb) can have long-term consequences. For example, Wiesner *et al.,* (2009) hypothesized that long-term influences on organisms may have an impact on their physiology and even their ability for reproduction. Furthermore, Lead (Pb) havebeen linked to alterations in gene expression in relation to different fish species (Lee *et.al.,* 2019) On the other hand, the exposed bivalve species *C. fluminea* showed notable mortality rates. Because they can offer precise and comprehensive information regarding the impact and bioavailability of chemicals, bivalve molluscs are frequently used in biomonitoring programmes and environmental perturbations (Zuykov *et al.,* 2013). This is because they are excellent water filters, digesting microalgae, bacteria, sediments, particulates, and natural nano-particles, and perhaps collecting various compounds in their tissues and being used as environmental indicators. (Write about fish and give fish reference not bivavlves) (Zuykov *et al.,* 2013)

The behaviour of Lead (Pb) after entering aquatic habitats harms organisms through a variety of harmful processes. For instance, fish, filter feeders, sediment dwellers of aquatic environments may be more susceptible to exposure and acute toxicity as a result dweller of the deposition and aggregation of Lead (Pb) aggregates in sediment. Our findings support the possibility that Lead (Pb) can enter fish through some organs like the gills or intestine (intestine not included in our thesis) that come into direct contact with heavy metal-contaminated water. Regarding other organisms, it has already been noted that Lead (Pb) can pierce mammalian skin, reaching various tissues and causing damages in various organs (Carocci, *et al.,* 2016). According to the various doses of Lead (Pb), histological investigation of the liver, gills, and kidney tissue from *C. puctatus* exhibited mild to severe structural changes including cytoplasmic vacuolation, hyperaemia, Melano macrophagecentres, fatty changes, congested blood vessels, dilated sinusoids, accumulation of Kupffer cells, mononuclear cells infiltration observed were also observed in livers from fish exposed to higher concentrations (30 to 90 milligrams per litter of Lead (Pb) (Only in higher concentrations). Overall, the findings indicate that exposure to sub-lethal

concentrations of Lead (Pb) causes a severe deterioration of hepatic tissues that finally leads in a localized or complete loss of tissue integrity (include all concentrations). Similar findings in juvenile carps exposed to Lead (Pb) concentrations between 10 and 20 milligrams per litter have also been reported by Hao *et al.,* (2009).

Fish that had their gills exposed showed a variety of alterations, such as varying degrees of hyperplasia (from minimal to total fusion of lamellae), as well as an increase in mucus secretion. Increased mucous found in bronchial tissues may serve as a barrier and defensive mechanism against external aggression because gills are in direct contact with the exposure medium, demonstrating the toxicity of Lead (Pb) to this organ. In order to protect the delicate gill epithelium from direct exposure to water contaminants, the gills frequently secrete mucus (Evans, D. H., 1987).

In the present study, animals exposed to the highest concentrations of Lead (Pb) of 10, 12, 24, 26milligrams per litter concentration of heavy metal were exposed for 5 and 10 days, and a histopathological examination of the kidney was observed. obtained results explained that on exposure to 26 milligrams per litter the increased glomerulus space, damaged glomerulus, increased tubular lumen, loss of first proximal tubule, patchy degeneration, separation of renal tubular epithelium from its basement, disorganized tubules, visceral membrane damage, red blood cells were observed. (Include all concentrations). The propensity of Lead (Pb) to produce free radicals, which result in oxidative stress and cell damage in living things, provides compelling evidence to explain why these particles are toxic. Aquatic species have a diverse pool of antioxidant enzymes that facilitate ROS detoxification, which is indicative of the antioxidants' effectiveness in controlling ROS generation. Superoxide dismutase (SOD), which changes oxygen (O_2) into hydrogen peroxide (H_2O_2) (Abele, *et al.,* 2011).

As a result, a multi-biomarker approach was applied in the current study to assess oxidative stress. SOD, CAT, and GST levels as well as lipid peroxidation levels in the target organs of *C. punctatus* demonstrate substantial variations over time between exposed organisms and controls. Results generally imply that species experienced oxidative stress as a result of exposure Lead (Pb). Numerous studies have shown that SOD activity increases as a first line of defence against oxidative stress in a variety of aquatic animals, including molluscs, crustaceans, polychaetas, and fish. SOD activity is therefore utilized to indicate the

ability to scavenge free radicals, demonstrating that the antioxidant defines mechanism is being overpowered by ROS (Monserrat, *et al.*, 2007). After 30 days of exposure to Lead (Pb), the results indicated a tendency for an increase in SOD enzymatic activity in the livers of *C. punctuates*. In compared to controls, a substantial increase in SOD activity ($p < 0.05$) was reported after 10 days. This increase may have been caused by the formation of new enzymes or by the up regulation of existing enzyme levels at lower concentrations. SOD activity increased from 5 to 10 days after exposure to Lead (Pb) at lower concentrations (10 and 26 milligrams per litter), but decreased from 5 to 10 days after exposure to organisms subjected to doses above 10 to 16 milligrams per litter. This downward trend in SOD activity suggests that the antioxidant defence mechanisms are being overworked and losing effectiveness.

GST is an enzyme found inside cells of phase II of foreign substances detoxification that aids in detoxification by triggering the binding of a variety of electrophilic foreign chemicals. By combining the byproducts of breakdown of lipid GSH it also helps safeguard cells from oxidative harm. As a result, GST is crucial in preventing oxidative stress-related damage to cells and tissues, whereas CAT is implicated in the oxidative reaction caused by stress by triggering a breakdown of H_2O_2 (Sule *et al.*, 2022). A drop in CAT levels is typically correlated with an increase in hydrogen peroxide and other oxyradicals that cause oxidative damage. In the current investigation, CAT and GST in the liver, gills, and kidney of *C. pucntats* followed a similar trend to SOD with a striking rise in enzyme activity at lower Lead (Pb) concentrations and a considerable decrease at higher concentrations. After 10 days of exposure to Lead (Pb) concentrations over 26 milligrams per litter, significant reductions in CAT and GST activities were typically observed in fish liver. (Farombi, *et al.,* 2007).

The findings imply that following an initial rise in antioxidant activity, the defence capacity eventually declines as a result of excessive ROS production and oxidative stress. At exposure concentrations of 24 and 26 mg/L, SOD activity in the digestive gland of *C. punctatus* showed distinct variations, with a trend toward rise followed by a trend toward decline at 10 days. While a significant rise in CAT and GST activities was observed in all treatments for exposure times of 30 and 60 days. *Juvenile Carp (Cyprinus carpio)* exposed to Lead (Pb) showed similar variations in SOD and CAT activities throughout concentration

and exposure duration (Hao *et al.,* 2009). According to the authors, since antioxidant enzymes are suppressed, ROS scavenging is decreased, and they may slowly build in fish's primary tissues. Additionally, some authors claim that while mild oxidative stress causes an increase in the synthesis of GSH and other enzymes like GST, catalase, and superoxide dismutase, severe oxidative stress can cause these enzymes' activities to be suppressed due to a lack of adaptive response mechanisms to induced stress (Davies, K. J., 2000).

Table 2 lists the values of the haematological parameters for each group of Lead (Pb) exposure to fish at various doses. In both exposure periods, red blood cells (RBCs), haemoglobin percentage (Hb), and haematocrit % (Hct) decreased significantly ($p < 0.05$) in the groups exposed to Lead (Pb). For these factors, the exposure period's major effect was significantly reduced ($P < 0.05$). The mean corpuscular value (MCV) significantly decreased ($p < 0.05$) as the dose of Lead (Pb) was increased. In all of the groups under observation, the mean corpuscular haemoglobin (MCH) did not demonstrate any appreciable variation. With increasing exposure to Lead (Pb) dosages, the mean corpuscular haemoglobin level (MCHC) showed a considerable rise.

Chapter-6

SUMMARY

Introduction

Water is a vital component of the Earth's hydrosphere and essential for all living things, including humans, plants, and animals. However, the constant deterioration of water bodies due to anthropogenic activities negatively impacts its intended use, such as the degradation of aquatic ecosystems and the use of contaminated water for irrigation, bathing, cleaning, or drinking. Heavy metal contamination of water bodies is a significant environmental issue due to their toxicity, bioaccumulation potential, and widespread distribution. Heavy metals found in nature, such as volcanic eruptions and deteriorating rocks, are also anthropogenic sources of heavy metals. In India, municipal sewage and industrial effluents contribute to the inclusion of heavy metals in soil and water. Human excrement contains heavy metals, such as copper, lead, nickel, and cadmium. Heavy metals are also found in household items like toothpaste, toothpaste, and some anti-dandruff shampoos. The dose, mode of exposure, chemical type, gender, age, genetics, and food intake of individuals exposed affect the level of toxicity of heavy metals.

Lead contamination is another significant issue, with heavy metals like lead (Pb) being the most toxic substance. Most Pb in the environment comes from mines, coal-fired power plants, ore smelting, batteries, industrial wastes, pesticides, and fuel additives. Lead has harmful impacts on both children and adults, including low blood levels, reduced intelligence, IQ, delays or damaged neurobehavioral growth, reduced hearing acuity, retarded growth, diminished attention span, and aggressive behavior in kids.

Conventional methods for reducing metal contamination include filtration, sedimentation, membrane filtration, and flotation. However, these methods have disadvantages, including cost, harmful by-products, and ineffective sequestration. Bioremediation, which uses organisms naturally to break down toxic/hazardous substances into less or non-toxic forms, is considered one of the safest, cleaner, and more environmentally conscious practices for decontaminating sites contaminated with pollutants.

Heavy metal pollution in aquatic lakes and rivers negatively impacts microbes, particularly fish, and is primarily caused by human operations such as crop food production, field erosion, and waste release. Metals like lead accumulate in aquatic living animals, including fish, and can contaminate fish's bodies, affecting their health and biological processes. The type of fish, concentration of the metals, and exposure duration all impact the toxicology of the metals. Fish with compromised neural systems are less able to interact with their surroundings due to heavy metal-mediated neurotoxicity.

The Gomti River, a tributary of the Ganges, is one of India's five transcendental rivers. It is a monsoon- and groundwater-fed river that travels through Uttar Pradesh and Lucknow, India. Pollution from sewage and industrial effluents has led to an increase in heavy metal pollution and deterioration of river water quality. Heavy metals are toxic and non-biodegradable, and the disposal of municipal and urban waste into river catchments in developing countries like India has become a significant concern in maintaining river water quality.

Regular monitoring and efficient management techniques for water quality of surface sources are crucial for the survival of living organisms. Constant monitoring of metal deposition in river water and sediments allows for the determination of river water acceptability for operations. To ensure the viability of riverine ecosystems, a long-term adaptation strategy considering environmental flows and ecosystem services is necessary.

This study aims to investigate the ability of toxicity of leadin fish. Channa punctatus, a predatory fish native to Asian freshwater environments, is a popular source of wholesome white meat. The study aims to determine the heavy metal concentration in Channa punctatus, a popular source of wholesome white meat consumed by a significant portion of the global population. The heavy metals' excessive dispersion into water bodies, such as plastic and electronic waste, can cause toxicity, leading to health issues such as cancer, skin ulcers, sinus carcinoma, pulmonary sensitization, itai-itai disease, renal failure, and mental impairment. The study aims to analyze the toxic effects of heavy metals on fish, the use of fish oil in herbal products, and the molecular pathways involved in heavy metal toxicity. The findings could help address the issue of heavy metal toxicity in the natural environment.

Review of literature

This chapter reviews past research on aquatic environment toxicity and Pb contamination, defining the toxicological impacts of contaminates on Channa punctatus morphology, behavior, histopathology, and biochemical parameters.

Methodology

In addition to a brief description of the techniques employed in the investigation of morphological, histological, behavioral, and biochemical characteristics, this section of the thesis covers experimental Techniques and Methods of Analysis. "Experimental Techniques and Methods of Analysis" refers to the methodology used for the study that incorporates the different approaches used to carry out the research plan.

Results

This chapter explores the morphological, histopathological, behavioral, and biochemical changes of Channa punctatus following different Pb doses, with regular observations. The study found that the schooling characteristic of this fish was weakened in Pb, leading to scale depletion, skin lesions, mucous accumulation, and gill clumping.

Exposure to Pb different doses in mg/l resulted in increased surfacing, air gulping, restlessness, erratic swimming, loose schooling, decreased activity, scattered fish, weaker response to food, hemorrhage, color fading, skin peeling, and rashes.

The liver of Channa punctatus becomes more fragile and darker in color after acute and chronic exposure to Pb, with no tumors or outgrowth observed. Hepatic cells undergo hypertrophy and polygonal shape loss. Fish exposed to Pb for 5 days showed no marked pathological changes. Kidneys in the Pb treated group showed degeneration of tubules and necrotic condition, with fused renal tubule cells and condensed cytoplasm. In 10 days, renal tubules undergo degeneration and hypertrophied cellular structure. Biochemical changes were observed in the tissues, including increased glucose levels, glycogen content, total protein levels, total lipid levels, and cholesterol levels.

Discussion

The literature in this field has been utilized to discuss potential modes of action.

Summary

The thesis summary is a comprehensive anthology that outlines the research work conducted in the research plan.

Pb is highly toxic to aquatic fauna, particularly fish, affecting their morphology, behavior, biochemical parameters, and histopathology. Different doses of Pb compound result in changes in fish morphology, behavior, and biochemical parameters, with liver and kidney structural changes. Therefore, it is crucial to treat water bodies and keep them free from heavy metals.

Bibliography

This is a collection of references used in research work, consulted at various text locations, as needed.

.

Chapter-7

CONCLUSION

The findings of this study are accordance with earlier investigations that suggested fish may be anaemic due to Lead (Pb). Previous research has shown that *Prochilodus lineatus* was become pale yellow after being exposed to $CuSO_4$. From our study, we may conclude that, Lead (Pb) when exposed to fish *C. punctatus* were it resulted in toxicity that included haematological alterations, organ abnormalities in gills, liver, and kidney as well as oxidative stress, inhibition (antioxidative enzymes get activated or inhibited in the study) of antioxidant activity, a rise in lipid peroxidation. The results of the trial revealed that neither the control group nor the treatment group experienced a noticeably different temperature or pH. The amount of dissolved oxygen in the water decreased steadily over time in all treatment groups, according to data collected after 5 and 10 days of Lead (Pb) exposure. The Control group's level of dissolved oxygen was slightly lower than that of the other treatment groups. In the treated group, had little dissolved oxygen? The findings demonstrated that when Lead (Pb) exposure dosages (10, 12, 24, 26 milligrams per litter) increased, nitrate and nitrite levels consistently rose but ammonia levels fell.

According to the experiment's findings, all groups of fish displayed uncoordinated movement, jerky movement, and stand-still zigzag swimming after being exposed to titanium oxide. The findings imply that discharges of Lead (Pb) into aquatic environments may constitute a risk to the wellbeing of animals and may have detrimental effects on aquatic life. It is obvious that the impacts of (HMs), notably Lead (Pb) on the environment are a matter of significant concern and that the precise mechanisms of toxicity of this and other types of Lead (Pb) must be understood since HMs are rapidly being used in daily life. Additionally, the release of HMs into freshwater ecosystems may increase the risk of human exposure through food and drinking supplies.

There are fewer researches concentrating on the aquatic animals that live in the sediment, despite an increase in studies demonstrating the toxicity of Lead (Pb) to aquatic organisms. This should raise serious concerns because the results of the current study imply that these organisms may be more vulnerable to Lead (Pb) exposure as a result of Lead (Pb)

deposition and accumulation in the sediment. And last, there is a pressing need for HM regulation. In order to assess the exposures and dangers related to HMs and safeguard human health and the environment, nanotoxicology data are necessary. When assessing the hazards of Heavy metals products along the entire food chain, it is important to take into account the special qualities and characteristics of Lead (Pb). The food and feed industry are predicted to be significantly impacted by heavy metals products in the future, with advantages for both the industry and the customer.

Chapter-8

REFERANCES

Abedi, M., Ghasemi, Y., & Nemati, M. M. (2024). Nanotechnology in toothpaste: Fundamentals, trends, and safety. *Heliyon*.

Abele, D., Vazquez-Medina, J. P., & Zenteno-Savin, T. (Eds.). (2011). *Oxidative stress in aquatic ecosystems*. John Wiley & Sons.

Abeysingha, N. S., Singh, M., Islam, A., & Sehgal, V. K. (2016). Climate change impacts on irrigated rice and wheat production in Gomti River basin of India: a case study. *Springer Plus*, *5*, 1-20.

Adekunle, I. M., & Akinyemi, M. F. (2004). Lead levels of certain consumer products in Nigeria: a case study of smoked fish foods from Abeokuta. *Food and Chemical Toxicology*, *42*(9), 1463-1468.

Adeyemo, T. (Ed.). (2010). *Africa Bible commentary: A one-volume commentary written by 70 African scholars*. Zondervan Academic.

Adkins-Jablonsky, S. J., Akscyn, R., Bennett, B. C., Roberts, Q., & Morris, J. J. (2020). Is community relevance enough? Civic and science identity impact of microbiology CUREs focused on community environmental justice. *Frontiers in Microbiology*, *11*, 578520.

Alvarado-Campo, K. L., Quintero, M., Cuadrado-Cano, B., Montoya-Giraldo, M., Otero-Tejada, E. L., Blandón, L., & Gómez-León, J. (2023). Heavy metal tolerance of microorganisms isolated from coastal marine sediments and their lead removal potential. Microorganisms, 11(11), 2708.

Adnan, M., Xiao, B., Ali, M. U., Xiao, P., Zhao, P., Wang, H., & Bibi, S. (2024). Heavy metals pollution from smelting activities: A threat to soil and groundwater. *Ecotoxicology and Environmental Safety*, *274*, 116189.

Agbugui, M. O., & Abe, G. O. (2022). Heavy metals in fish: bioaccumulation and health. *British Journal of Earth Sciences Research*, *10*(1), 47-66.

Ahmad, P., Sarwat, M., Bhat, N. A., Wani, M. R., Kazi, A. G., & Tran, L. S. P. (2015). Alleviation of cadmium toxicity in Brassica junceaL.(Czern. &Coss.) by calcium

application involves various physiological and biochemical strategies. *PloS one*, *10*(1), e0114571.

Ajala, L. O., Obasi, N. A., Fasuan, T. O., Ominyi, C. E., &Onwukeme, V. I. (2024). Ecotoxicological Risks in Floodplain Sediments: Linking Current Conditions to Future Threats. *Scientific African*, e02201.

Alam, M., Rohani, M. F., & Hossain, M. S. (2023). Heavy metals accumulation in some important fish species cultured in commercial fish farm of Natore, Bangladesh and possible health risk evaluation. *Emerging Contaminants*, *9*(4), 100254.

Ali, A. S., & US SA, A. R. (2014). Effect of different heavy metal pollution on fish. *Res. J. Chem. Environ. Sci*, *2*(1), 74-79.

Ali, M. M., Hossain, D., Al-Imran, A., Khan, M. S., Begum, M., & Osman, M. H. (2021). Environmental pollution with heavy metals: A public health concern. *Heavy metals-their environmental impacts and mitigation*, 771-783.

Aljohani, N. S., Kavil, Y. N., Alelyani, S. S., Al-Farawati, R. K., Orif, M. I., Aljohani, N. H., & Salam, M. A. (2023). Bioaccumulation of heavy metals in fish collected from the Eastern Coast of Saudi Arabia and Human Health Implications. *Regional Studies in Marine Science*, *62*, 102986.

Alloway, B. J. (Ed.). (2012). *Heavy metals in soils: trace metals and metalloids in soils and their bioavailability* (Vol. 22). Springer Science & Business Media.

Amuda, O. S., Giwa, A., & Bello, I. A. (2007). Removal of heavy metal from industrial wastewater using modified activated coconut shell carbon. *Biochemical Engineering Journal*, *36*(2), 174-181.

Arena, A., Zoccali, M., Trozzi, A., Tranchida, P. Q., & Mondello, L. (2021). Occurrence of mineral oil hydrocarbons in omega-3 fatty acid dietary supplements. *Foods*, *10*(10), 2424.

Arslan, K., & Akbaba, G. B. (2020). In vitro genotoxicity assessment and comparison of cerium (IV) oxide micro-and nanoparticles. Toxicology and Industrial Health, 36(2), 76-83.

Arthington, Á. H., Naiman, R. J., Mcclain, M. E., & Nilsson, C. (2010). Preserving the biodiversity and ecological services of rivers: new challenges and research opportunities. *Freshwater biology*, *55*(1), 1-16.

Azar, H., & Vajargah, M. F. (2023). Investigating the effects of accumulation of lead and cadmium metals in fish and its impact on human health. *J Aquac Mar Biol*, *12*(2), 209-213.

Barbosa Jr, F., Tanus-Santos, J. E., Gerlach, R. F., & Parsons, P. J. (2005). A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. *Environmental health perspectives*, *113*(12), 1669-1674.

Behra, R., & Krug, H. (2008). Nanoparticles at large. *Nature nanotechnology*, *3*(5), 253-254.

Bellinger, D. C. (2005). Teratogen update: lead and pregnancy. *Birth Defects Research Part A: Clinical and Molecular Teratology*, *73*(6), 409-420.

Bind, A., Kushwaha, A., Devi, G., Goswami, S., Sen, B., & Prakash, V. (2019). Biosorption valorization of floating and submerged macrophytes for heavy-metal removal in a multi-component system. *Applied Water Science*, *9*, 1-9.

Borgese, L., Federici, S., Zacco, A., Gianoncelli, A., Rizzo, L., Smith, D. R., ... & Bontempi, E. (2013). Metal fractionation in soils and assessment of environmental contamination in Vallecamonica, Italy. *Environmental Science and Pollution Research*, *20*, 5067-5075.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.

Budi, H. S., Catalan Opulencia, M. J., Afra, A., Abdelbasset, W. K., Abdullaev, D., Majdi, A., & Mohammadi, M. J. (2024). Source, toxicity and carcinogenic health risk assessment of heavy metals. *Reviews on Environmental Health*, *39*(1), 77-90.

Burke, N. D., Nixon, B., Roman, S. D., Schjenken, J. E., Walters, J. L., Aitken, R. J., & Bromfield, E. G. (2022). Male infertility and somatic health insights into lipid damage as a mechanistic link. *Nature Reviews Urology*, *19*(12), 727-750.

Buzukashvili, S., Sommerville, R., Rowson, N. A., & Waters, K. E. (2024). An overview of zeolites synthesised from coal fly ash and their potential for extracting heavy metals from industrial wastewater. *Canadian Metallurgical Quarterly*, *63*(1), 130-152.

Caill-Milly, N., Sanchez, F., Benito, D., Ruiz, P., Izagirre, U., &Briaudeau, T. (2023). Assessment of size at first maturity for Ruditapesphilippinarum from Arcachon Bay

(French Atlantic coast): New insights for fishery management. *Estuarine, Coastal and Shelf Science*, *285*, 108321.

Carocci, A., Catalano, A., Lauria, G., Sinicropi, M. S., & Genchi, G. (2016). Lead toxicity, antioxidant defense and environment. *Reviews of environmental contamination and toxicology*, 45-67.

Chatterjee, A. K., Sarkar, R. K., Chattopadhyay, A. P., Aich, P., Chakraborty, R., & Basu, T. (2012). A simple robust method for synthesis of metallic copper nanoparticles of high antibacterial potency against E. coli. *Nanotechnology*, *23*(8), 085103.

Chawla, H., Singh, S. K., &Haritash, A. K. (2024). Reversing the damage: ecological restoration of polluted water bodies affected by pollutants due to anthropogenic activities. *Environmental Science and Pollution Research*, *31*(1), 127-143.

Chen, J., Chen, Y., Liu, W., Bai, C., Liu, X., Liu, K., & Huang, C. (2012). Developmental lead acetate exposure induces embryonic toxicity and memory deficit in adult zebrafish. *Neurotoxicology and teratology*, *34*(6), 581-586.

Chen, Q., Zhao, H., Liu, Y., Jin, L., & Peng, R. (2023). Factors affecting the adsorption of heavy metals by microplastics and their toxic effects on fish. *Toxics*, *11*(6), 490.

Cheng, H., & Hu, Y. (2010). Municipal solid waste (MSW) as a renewable source of energy: Current and future practices in China. *Bioresource technology*, *101*(11), 3816-3824.

Davies, K. J. (2000). Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. *IUBMB life*, *50*(4-5), 279-289.

de Mendonça Francisco, C., Pavanin, L. A., Morelli, S., Bravo, J. V. M., & Pereira, B. B. (2023). Using native fish in eco-genotoxic assessment of heavy metal contamination pollution arising from nearby large Brazilian rivers. *Journal of Toxicology and Environmental Health, Part A*, *86*(2-3), 74-85.

Djedjibegovic, J., Marjanovic, A., Tahirovic, D., Caklovica, K., Turalic, A., Lugusic, A., ... & Caklovica, F. (2020). Heavy metals in commercial fish and seafood products and risk assessment in adult population in Bosnia and Herzegovina. *Scientific Reports*, *10*(1), 1-8.

Dos Santos, H. D. A. G., Kitamura, R. S. A., Soares, G. D. C. B., Dos Santos, M. P., de Souza Miranda, L. P., Mela, M., & de Assis, H. C. S. (2024). Assessing the water

quality in a World Heritage Site using biomarkers in top fish predators. *Science of The Total Environment*, 172072.

Dunier, M. (1996). Water pollution and immunosuppression of freshwater fish. *Italian Journal of Zoology*, *63*(4), 303-309.

Elumalai, S., Prabhu, K., Selvan, G. P., & Ramasamy, P. (2023). Review on heavy metal contaminants in freshwater fish in South India: current situation and future perspective. *Environmental Science and Pollution Research*, *30*(57), 119594-119611.

Eroglu, A., Dogan, Z., Kanak, E. G., Atli, G. Ü. L. İ., & Canli, M. U. S. T. A. F. A. (2015). Effects of heavy metals (Cd, Cu, Cr, Pb, Zn) on fish glutathione metabolism. *Environmental Science and Pollution Research*, *22*, 3229-3237.

Esmaeili, N. (2021). Blood performance: a new formula for fish growth and health. *Biology*, *10*(12), 1236.

Evans, D. H. (1987). The fish gill: site of action and model for toxic effects of environmental pollutants. *Environmental health perspectives*, *71*, 47-58.

Farombi, E. O., Adelowo, O. A., &Ajimoko, Y. R. (2007). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African cat fish (Clarias gariepinus) from Nigeria Ogun River. *International journal of Environmental research and Public health*, *4*(2), 158-165.

Fritioff, Å., & Greger, M. (2006). Uptake and distribution of Zn, Cu, Cd, and Pb in an aquatic plant Potamogetonnatans. *Chemosphere*, *63*(2), 220-227.

Godse, G., & Godse, K. (2024). Safety, Efficacy and Attributes of 2.5% Selenium Sulfide Shampoo in the Treatment of Dandruff: A Single-Center Study. *Cureus*, *16*(3).

Goldhirsch, A., Wood, W. C., Coates, A. S., Gelber, R. D., Thürlimann, B., & Senn, H. J. (2011). Strategies for subtypes dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Annals of oncology*, *22*(8), 1736-1747.

Gomez Isaza, D. F., Cramp, R. L., & Franklin, C. E. (2021). Exposure to nitrate increases susceptibility to hypoxia in fish. *Physiological and Biochemical Zoology*, *94*(2), 124-142.

Green, A. J., & Planchart, A. (2018). The neurological toxicity of heavy metals: A fish perspective. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *208*, 12-19.

Habib, S. S., Batool, A. I., Rehman, M. F. U., & Naz, S. (2023). Assessment and bioaccumulation of heavy metals in fish feeds, water, and some tissues of Cyprinus carpio cultured in different environments (Biofloc Technology and Earthen Pond System). *Biological Trace Element Research*, *201*(7), 3474-3486.

Habib, S. S., Naz, S., Fazio, F., Cravana, C., Ullah, M., Rind, K. H., & Khayyam, K. (2024). Assessment and bioaccumulation of heavy metals in water, fish (wild and farmed) and associated human health risk. *Biological Trace Element Research*, *202*(2), 725-735.

Habig, W. H., Pabst, M. J., Fleischner, G., Gatmaitan, Z., Arias, I. M., & Jakoby, W. B. (1974). The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. *Proceedings of the National Academy of Sciences*, *71*(10), 3879-3882.

Hao, Y., Chen, L., Zhang, X., Zhang, D., Zhang, X., Yu, Y., & Fu, J. (2013). Trace elements in fish from Taihu Lake, China: Levels, associated risks, and trophic transfer. *Ecotoxicology and environmental safety*, *90*, 89-97.

Harshitha, P., Bose, K., & Dsouza, H. S. (2024). Influence of lead-induced toxicity on the inflammatory cytokines. *Toxicology*, *503*, 153771.

Holmstrup, M., Bindesbøl, A. M., Oostingh, G. J., Duschl, A., Scheil, V., Köhler, H. R., ... & Spurgeon, D. J. (2010). Interactions between effects of environmental chemicals and natural stressors: a review. *Science of the Total Environment*, *408*(18), 3746-3762.

Jadaa, W. (2024). Wastewater Treatment Utilizing Industrial Waste Fly Ash as a Low-Cost Adsorbent for Heavy Metal Removal: Literature Review. *Clean Technologies*, *6*(1), 221-279.

Jagaba, A. H., Lawal, I. M., Birniwa, A. H., Affam, A. C., Usman, A. K., Soja, U. B., & Yaro, N. S. A. (2024). Sources of Water Contamination by Heavy Metals. In *Membrane Technologies for Heavy Metal Removal from Water* (pp. 3-27). CRC Press.

Jain, R. Water Quality Assessment of River Gomti in Lucknow City.

Jamil Emon, F., Rohani, M. F., Sumaiya, N., Tuj Jannat, M. F., Akter, Y., Shahjahan, M., & Goh, K. W. (2023). Bioaccumulation and bioremediation of heavy metals in fishes-A review. *Toxics*, *11*(6), 510.

Javed, M., & Usmani, N. (2019). An overview of the adverse effects of heavy metal contamination on fish health. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *89*, 389-403.

K AL Taee, S., Al-Mallah, K. H., & Kh Ismail, H. (2020). Review on some heavy metals toxicity on freshwater fishes. *Journal of Applied Veterinary Sciences*, *5*(3), 78-86.

Kakade, A., Sharma, M., Salama, E. S., Zhang, P., Zhang, L., Xing, X., & Li, X. (2023). Heavy metals (HMs) pollution in the aquatic environment: Role of probiotics and gut microbiota in HMs remediation. *Environmental research*, *223*, 115186.

Karri, V., Schuhmacher, M., & Kumar, V. (2016). Heavy metals (Pb, Cd, As and Me Hg) as risk factors for cognitive dysfunction: A general review of metal mixture mechanism in brain. *Environmental toxicology and pharmacology*, *48*, 203-213.

Kato, S., Chmielewski, M., Honda, H., Pecoits-Filho, R., Matsuo, S., Yuzawa, Y., & Lindholm, B. (2008). Aspects of immune dysfunction in end-stage renal disease. *Clinical Journal of the American Society of Nephrology*, *3*(5), 1526-1533.

Kavitha, T., & Sridharan, D. (2010). Security vulnerabilities in wireless sensor networks: A survey. *Journal of information Assurance and Security*, *5*(1), 31-44.

Kay, J. E., Brody, J. G., Schwarzman, M., & Rudel, R. A. (2024). Application of the key characteristics framework to identify potential breast carcinogens using publicly available in vivo, in vitro, and in silico data. *Environmental Health Perspectives*, *132*(1), 017002.

Khan, S. A., Liu, X., & Shah, B. R. (2014). Impact of acute toxicity of lead acetate on the level of essential trace metals and histopathological changes in Crucian carp.(Carassius auratus gibelio). *JAPS: Journal of Animal & Plant Sciences*, *24*(5).

Kim, D. Y., Jeon, H., & Shin, H. S. (2023). Risk Assessment and Determination of Arsenic and Heavy Metals in Fishery Products in Korea. *Foods*, *12*(20), 3750.

Krishan, A., Khursheed, A., & Mishra, R. K. (2022). Evaluation of water quality using water quality index, synthetic pollution index, and GIS technique: a case study of the river

Gomti, Lucknow, India. *Environmental Science and Pollution Research*, *29*(54), 81954-81969.

Kumar, A., Kumar, A., MMS, C. P., Chaturvedi, A. K., Shabnam, A. A., Subrahmanyam, G., & Yadav, K. K. (2020). Lead toxicity: health hazards, influence on food chain, and sustainable remediation approaches. *International journal of environmental research and public health*, *17*(7), 2179.

Kumar, A., Kumar, P., Raj, A., & Kisku, G. C. (2024). Assessment of influence of heavy metal, organochlorine pesticide, and bacterial presence on water quality of Gomti river, India. *Environment, Development and Sustainability*, *26*(1), 1879-1901.

Kumar, D., & Desai, K. (2011). Pollution abatement in milk dairy industry. *Journal of Current Pharma Research*, *1*(2), 145.

Kumar, V., Sharma, N., Sharma, P., Pasrija, R., Kaur, K., Umesh, M., & Thazeem, B. (2023). Toxicity analysis of endocrine disrupting pesticides on non-target organisms: A critical analysis on toxicity mechanisms. *Toxicology and Applied Pharmacology*, *474*,116623.

Kurade, M. B., Saha, S., Salama, E. S., Patil, S. M., Govindwar, S. P., & Jeon, B. H. (2019). Acetoclastic methanogenesis led by Methanosarcina in anaerobic co-digestion of fats, oil and grease for enhanced production of methane. *Bioresource technology*, *272*, 351-359.

Lee, J. W., Choi, H., Hwang, U. K., Kang, J. C., Kang, Y. J., Kim, K. I., & Kim, J. H. (2019). Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. *Environmental toxicology and pharmacology*, *68*, 101-108.

Lee, L. S. (2022). Quantitative Tools for the Prediction of Pavement Damages Associated with Urban Trees. *Arboriculture & Urban Forestry*, *48*(4).

Lenart-Boroń, A., &Boroń, P. (2014). *The effect of industrial heavy metal pollution on microbial abundance and diversity in soils-a review*. IntechOpen.

Li, Y., Zhou, X., Guo, W., Fu, Y., Ruan, G., Fang, L., & Wang, Q. (2023). Effects of lead contamination on histology, antioxidant and intestinal microbiota responses in freshwater crayfish, Procambarusclarkii. *Aquatic Toxicology*, *265*, 106768.

Lin, C. C., & Lin, H. L. (2005). Remediation of soil contaminated with the heavy metal ($Cd2^{+}$). *Journal of hazardous materials*, *122*(1-2), 7-15.

Linhua, H. A. O., Zhenyu, W. A. N. G., &Baoshan, X. I. N. G. (2009). Effect of sub-acute exposure to TiO2 nanoparticles on oxidative stress and histopathological changes in Juvenile Carp (Cyprinus carpio). *Journal of Environmental Sciences*, *21*(10), 1459-1466.

Liu, M., Xu, Y., Nawab, J., Rahman, Z., Khan, S., Idress, M., ... & Li, G. (2020). Contamination features, geo-accumulation, enrichments and human health risks of toxic heavy metal (loids) from fish consumption collected along Swat river, Pakistan. *Environmental Technology & Innovation*, *17*, 100554.

Łuczyńska, J., Pietrzak-Fiećko, R., Purkiewicz, A., & Łuczyński, M. J. (2022). Assessment of fish quality based on the content of heavy metals. *International Journal of Environmental Research and Public Health*, *19*(4), 2307.

MacKinnon, D. F., & Chen, L. N. (2024). Depressive disorders. *Tasman's Psychiatry*, 1-58.

Manara, A. (2012). Plant responses to heavy metal toxicity. *Plants and heavy metals*, 27-53.

Margot, J., Rossi, L., Barry, D. A., & Holliger, C. (2015). A review of the fate of micropollutants in wastewater treatment plants. *Wiley Interdisciplinary Reviews: Water*, *2*(5), 457-487.

Marlatt, V. L., Bayen, S., Castaneda-Cortès, D., Delbès, G., Grigorova, P., Langlois, V. S., ... & Van Der Kraak, G. (2022). Impacts of endocrine disrupting chemicals on reproduction in wildlife and humans. *Environmental Research*, *208*, 112584.

Masindi, V., &Muedi, K. L. (2018). Environmental contamination by heavy metals. *Heavy metals*, *10*(4), 115-133.

Mehar, S., Anam, I., Masood, Z., Alvi, S., Khan, W., Kabir, M., & Khan, T. (2023). Bioaccumulation of heavy metals in the different tissues of Mackerel scad, Decapterusmacarellus (Cuvier, 1833) collected from Karachi and Gwadar Coasts of Pakistan. *Saudi Journal of Biological Sciences*, *30*(2), 103540.

Metryka, E., Chibowska, K., Gutowska, I., Falkowska, A., Kupnicka, P., Barczak, K., & Baranowska-Bosiacka, I. (2018). Lead (Pb) exposure enhances expression of factors associated with inflammation. *International journal of molecular sciences*, *19*(6), 1813.

Mielke, A. (2011). A gradient structure for reaction–diffusion systems and for energy-drift-diffusion systems. *Nonlinearity*, *24*(4), 1329.

Mohammed Abdulridha, J. A. (2016). *Toxicity assessment and bioaccumulation of heavy metals in red tilapia/Mohammed Abdulridha Jasim Aldoghachi* (Doctoral dissertation, University of Malaya).

Mohanakavitha, T., Shankar, K., Divahar, R., Meenambal, T., & Saravanan, R. (2019). Impact of industrial wastewater disposal on surface water bodies in Kalingarayan canal, Erode district, Tamil Nadu, India. *Archives of Agriculture and Environmental Science*, *4*(4), 379-387.

Moiseenko, T. I., & Gashkina, N. A. (2020). Distribution and bioaccumulation of heavy metals (Hg, Cd and Pb) in fish: Influence of the aquatic environment and climate. *Environmental Research Letters*, *15*(11), 115013.

Monserrat, J. M., Martínez, P. E., Geracitano, L. A., Amado, L. L., Martins, C. M. G., Pinho, G. L. L., & Bianchini, A. (2007). Pollution biomarkers in estuarine animals: critical review and new perspectives. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *146*(1-2), 221-234.

Nishijo, M., Nakagawa, H., Suwazono, Y., Nogawa, K., & Kido, T. (2017). Causes of death in patients with Itai-itai disease suffering from severe chronic cadmium poisoning: a nested case–control analysis of a follow-up study in Japan. *BMJ open*, *7*(7), e015694.

Norani, E., Haghparast, S., Raeisi, H., & Bastami, K. D. (2023). A spatiotemporal study on contamination and bioaccumulation of heavy metals in sediment and cyprinid fish (Capoetarazii) from Telar River, Iran. *Marine Pollution Bulletin*, *194*, 115421.

Nwani, C. D., Nagpure, N. S., Kumar, R., Kushwaha, B., Kumar, P., & Lakra, W. S. (2010). Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish Channa punctatus (Bloch).

Pandi Prabha, S., & Rajkumar, J. (2015). Histological study on protective effect of aquatic weed Hydrilla verticillata against lead induced toxicity in fish. International Journal of Pharmacy and Pharmaceutical Sciences, 7(8), 132-136.

Patchaiyappan, A., Arulkumar, A., Shynshiang, K., Anandkumar, A., Prabakaran, K., Basu, A., & Devipriya, S. (2023). Bioaccumulation of heavy metals in commercially

important marine species from Puducherry coast, Southeast India. *Regional Studies in Marine Science*, *65*, 103080.

Patel, J., Patel, K., Mudgal, A., Panchal, H., &Sadasivuni, K. K. (2020). Experimental investigations of atmospheric water extraction device under different climatic conditions. *Sustainable Energy Technologies and Assessments*, *38*, 100677.

Patel, M., Patel, S. S., Kumar, P., Mondal, D. P., Singh, B., Khan, M. A., & Singh, S. (2021). Advancements in spontaneous microbial desalination technology for sustainable water purification and simultaneous power generation: a review. *Journal of Environmental Management*, *297*, 113374.

Paul, N., Chakraborty, S., & Sengupta, M. (2014). Lead toxicity on non-specific immune mechanisms of freshwater fish Channa punctatus. *Aquatic Toxicology*, *152*, 105-112.

Prabakaran, K., Sompongchaiyakul, P., Bureekul, S., Wang, X., & Charoenpong, C. (2024). Heavy metal bioaccumulation and risk assessment in fishery resources from the Gulf of Thailand. *Marine Pollution Bulletin*, *198*, 115864.

Prabha, S. P., Rajkumar, J., Suresh, S., & Karthikeyan, S. (2018). Antiosteoporotic effect of Hydrilla verticillata against lead induced damage in bone samples of edible fish Labeorohita an FTIR approach. *Materials Science and Engineering: C*, *90*, 657-663.

Rakib, M. R. J., Jolly, Y. N., Enyoh, C. E., Khandaker, M. U., Hossain, M. B., Akther, S., ... & Bradley, D. A. (2021). Levels and health risk assessment of heavy metals in dried fish consumed in Bangladesh. *Scientific reports*, *11*(1), 14642.

Raza, B., Javed, M., Ambreen, F., & Latif, F. (2016). Toxic effect of lead chloride on antioxidant enzyme in the liver and kidney of fish. *Journal of Bioresource Management*, *3*(4), 1.

Rehman, T., Naz, S., Hussain, R., Chatha, A. M. M., Ahmad, F., Yamin, A., ... & Shaheen, A. (2021). Exposure to heavy metals causes histopathological changes and alters antioxidant enzymes in fresh water fish (Oreochromis niloticus).

Rehman, Z., Junaid, M. F., Ijaz, N., Khalid, U., & Ijaz, Z. (2023). Remediation methods of heavy metal contaminated soils from environmental and geotechnical standpoints. *Science of The Total Environment*, *867*, 161468.

Rogers, J. T. (2003). *A mechanistic approach to acute lead toxicity in the rainbow trout: Investigations of lead-induced ionoregulatory disruption* (Doctoral dissertation).

Rotimi, D. E., & Singh, S. K. (2024). Implications of lifestyle factors on male reproductive health. *JBRA Assisted Reproduction*.

Rubin, R., Strayer, D. S., & Rubin, E. (Eds.). (2008). *Rubin's pathology: clinicopathologic foundations of medicine*. Lippincott Williams & Wilkins.

Sadeghi, P., Loghmani, M., & Frokhzad, S. (2020). Human health risk assessment of heavy metals via consumption of commercial marine fish (Thunnus albacares, Euthynnus affinis, and Katsuwonus pelamis) in Oman Sea. *Environmental science and pollution research*, *27*, 14944-14952.

Sahoo, S., & Goswami, S. (2024). Theoretical framework for assessing the economic and environmental impact of water pollution: A detailed study on sustainable development of India. *Journal of Future Sustainability*, *4*(1), 23-34.

Sankhla, M. S., Kumari, M., Nandan, M., Kumar, R., & Agrawal, P. (2016). Heavy metals contamination in water and their hazardous effect on human health-a review. *Int. J. Curr. Microbiol. App. Sci (2016)*, *5*(10), 759-766.

Seibel, H., Baßmann, B., &Rebl, A. (2021). Blood will tell: what hematological analyses can reveal about fish welfare. *Frontiers in Veterinary Science*, *8*, 616955.

Shahjahan, M., Taslima, K., Rahman, M. S., Al-Emran, M. D., Alam, S. I., & Faggio, C. (2022). Effects of heavy metals on fish physiology–a review. *Chemosphere*, *300*, 134519.

Sharma, M. (2005). Sustainable Development: Prism of Developing Countries. *Environment: Problems And Policies:(encyclopaedia Of Environment)*, 105.

Sharma, P., Jha, A. B., & Dubey, R. S. (2019). Oxidative stress and antioxidative defense system in plants growing under abiotic stresses. In *Handbook of Plant and Crop Stress, Fourth Edition* (pp. 93-136). CRC press.

Sharma, V., & Mehdi, M. M. (2023). Oxidative stress, inflammation and hormesis: The role of dietary and lifestyle modifications on aging. *Neurochemistry international*, *164*, 105490.

Sheikhzadeh, H., &Hamidian, A. H. (2021). Bioaccumulation of heavy metals in fish species of Iran: a review. *Environmental Geochemistry and Health*, *43*(10), 3749-3869.

Shi, X., Chen, Z., Wang, H., Yeung, D. Y., Wong, W. K., & Woo, W. C. (2015). Convolutional LSTM network: A machine learning approach for precipitation nowcasting. *Advances in neural information processing systems*, *28*.

Singh, A., & Kostova, I. (2024). Health effects of heavy metal contaminants Vis-à-Vis microbial response in their bioremediation. *Inorganica Chimica Acta*, 122068.

Singh, D., Nath, K., Trivedi, S. P., & Sharma, Y. K. (2008). Impact of copper on haematological profile of freshwater fish, Channa punctatus. *Journal of Environmental biology*, *29*(2), 253.

Singh, K. K., Tewari, G., & Kumar, S. (2020). Evaluation of groundwater quality for suitability of irrigation purposes: a case study in the Udham Singh Nagar, Uttarakhand. *Journal of Chemistry*, *2020*, 1-15.

Sirisangarunroj, P., Monboonpitak, N., Karnpanit, W., Sridonpai, P., Singhato, A., Laitip, N., ... & Judprasong, K. (2023). Toxic Heavy Metals and Their Risk Assessment of Exposure in Selected Freshwater and Marine Fish in Thailand. *Foods*, *12*(21), 3967.

Sonone, S. S., Jadhav, S., Sankhla, M. S., & Kumar, R. (2020). Water contamination by heavy metals and their toxic effect on aquaculture and human health through food Chain. *Lett. Appl. NanoBioScience*, *10*(2), 2148-2166.

Steenland, K., &Boffetta, P. (2000). Lead and cancer in humans: where are we now?. *American journal of industrial medicine*, *38*(3), 295-299.

Sule, R. O., Condon, L., & Gomes, A. V. (2022). A common feature of pesticides: oxidative stress the role of oxidative stress in pesticide-induced toxicity. *Oxidative medicine and cellular longevity*, *2022*.

Taslima, K., Al-Emran, M., Rahman, M. S., Hasan, J., Ferdous, Z., Rohani, M. F., & Shahjahan, M. (2022). Impacts of heavy metals on early development, growth and reproduction of fish–A review. *Toxicology reports*, *9*, 858-868.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology: volume 3: environmental toxicology*, 133-164.

Trivedy, A., & Khatun, M. (2024). Water, Sanitation, and Hygiene (WASH) condition in West Bengal, India: Exploring geospatial inequality, patterns, and determinants. *GeoJournal*, *89*(1), 1-18.

Ture, M., Kilic, M. B., & Altinok, I. (2021). Relationship between heavy metal accumulation in fish muscle and heavy metal resistance genes in bacteria isolated from fish. *Biological Trace Element Research*, *199*(4), 1595-1603.

Ukaogo, P. O., Ewuzie, U., & Onwuka, C. V. (2020). Environmental pollution: causes, effects, and the remedies. In *Microorganisms for sustainable environment and health* (pp. 419-429). Elsevier.

World Health Organization. (2016). Protecting surface water for health: identifying, assessing and managing drinking-water quality risks in surface-water catchments.

Xiong, W., MacIsaac, H. J., & Zhan, A. (2024). An overlooked source of false positives in eDNA-based biodiversity assessment and management. *Journal of Environmental Management*, *358*, 120949.

Xu, J., Li, Y., Wang, S., Long, S., Wu, Y., & Chen, Z. (2023). Sources, transfers and the fate of heavy metals in soil-wheat systems: The case of lead (Pb)/zinc (Zn) smelting region. *Journal of Hazardous Materials*, *441*, 129863.

Yilmaz, B., Terekeci, H., Sandal, S., & Kelestimur, F. (2020). Endocrine disrupting chemicals: exposure, effects on human health, mechanism of action, models for testing and strategies for prevention. *Reviews in endocrine and metabolic disorders*, *21*, 127-147.

Zelikoff, J. T. (1993). Metal pollution-induced immunomodulation in fish. *Annual Review of Fish Diseases*, *3*, 305-325.

Zhang, P., Yang, M., Lan, J., Huang, Y., Zhang, J., Huang, S., & Ru, J. (2023). Water quality degradation due to heavy metal contamination: health impacts and eco-friendly approaches for heavy metal remediation. *Toxics*, *11*(10), 828.

Zheng, X., Wei, C., Fan, J., Liu, X., Hou, Y., Ling, J., & Liu, P. (2023). Acute Toxicity Assessment and Prediction Models of Four Heavy Metals. *Toxics*, *11*(4), 346.

Zou, X., Li, P., Huang, Q., & Zhang, H. (2016). The different response mechanisms of Wolffia globosa: Light-induced silver nanoparticle toxicity. *Aquatic toxicology*, *176*, 97-105.

Zuykov, M., Pelletier, E., & Harper, D. A. (2013). Bivalve mollusks in metal pollution studies: from bioaccumulation to biomonitoring. *Chemosphere*, *93*(2), 201-208.

Curriculum Vitae

Name	:	Bharti Gupta
Father's Name	:	Shri Ram Kumar Gupta
Date of Birth	:	18/05/1996
Marital status	:	Unmarried
Nationality	:	Indian
Language known	:	Hindi and English
Permanent address	:	Civil Line Ayodhya, Naka Hanuman Ghari, Faizabad (Uttar Pradesh) Pin code-224001
Mobile No.	:	+91 9580654160
Email Id.	:	*bg6568053@gmail.com*

Academic Credentials

Class	Board/University	Subject	Year	Division
High School	U.P. Board	Science	2012	1st
Intermediate	U.P. Board	Science	2014	1st
B.Sc.	K.S.Saket P.G. College, Ayodhya, Faizabad (UP) India	ZBC	2017	1st
M.Sc.	K.S.Saket P.G. College, Ayodhya, Faizabad (UP) India	Zoology	2019	1st
Ph.D.	Maharishi University of Information Technology, Lucknow (UP) India	Zoology	Pursuing	

DECLARATION

I hereby declare that all the information furnished above is true to the best of my knowledge & belief, documentary evidence will support them as and when required.

Date: / / 2024 **(Bharti Gupta)**

Place: Ayodhya

List of Publications
(Journal and conference in separate list)

1. Abstract

a.) Sarayu River heavy metal poisoning on the histopathological alteration in various organs of Channa punctatus (IEEC/2023/ABS/4.7/360)

b.) Control and prevention of infectious disease of fish

2. Conferance Certificate

a.) IEEC BHU -2023 on Innovation Application in Agricultural Extension for Sustainable Food & Environmental Education Conference, BHU, Varanasi (UP) India, during January 27-30, 2023.

b.) National Conference, Current Treands in Biological Sciences for Sustainable Agriculture Environment & Health Under Climate Change, XV Convention of the Indian Society of Agricultural Biochemists, Lucknow University on during Nov. 23-25, 2023

3. Review Article

Gupta, B., Maurya, R. (2024). Metalliferous uptake and Deposition in Fish in contaminated environments, Journal of Wildlife and Biodiversity, 8(2), 103-121. DOI: https://doi.org/10.5281/zenodo.

4. Research Paper

Gupta, B., Maurya, R. (2024). A comparative study of acquisition of heavy metals and its toxicological effects in fish species: Siluriformes and Channa punctata. DOI: https://doi.org/10.47815/apsr.2024.10364

and biochemical parameters of Indian mustard (Brassica juncea L.). This annual herb originates from natural hybridization between black mustard (Brassica nigra L. Koch) and turnip mustard (Brassica rapa L.) retaining the whole genome of both parents, therefore it is amphidiploid. Mustard oil is harmful because of its high allyl isothiocyanate content. Fresh seeds or mustard powder do not possess essential oil and hence preparation made from these do not contain allyl isothiocyanate.

Keywords: PGPR, Rhizosphere, Endophytic bacteria, Abiotic stress, Bio-fertilizers, Biocontrol, Sustainable agriculture.

IEEC/2023/ABS/4.7/360

Sarayu River heavy metal poisoning on the histopathological alterations in various organs of Channa punctatus

Bharti Gupta[a], Ramakant Maurya[b]

[a,b]Deparment of Zoology, School of science, Maharshi University of Information Technology, Lucknow, India

e-mail: bg6568053@gmail.com

The goal of the present research was to assess how the biomagnification of metals (Zn, Cu, and Hg) in the gills, kidneys, liver, and muscle of Channa punctatus varied over the year. In the wintertime, summertime, springtime, and fall seasons, 50 fish samples (average wet body weight: 450.77 ±5.12 g) were taken from the sarayu. River in Ayodhya. Atomic absorption analysis was used to evaluate the heavy metals. The results of this investigation showed that metal bioaccumulation varies seasonally. The lowest concentrations of Zn, Cu, and Hg were found during the winter season, whereas the highest concentrations were found during the summer.Zn and Hg were bio accumulated in the following order in the organs: gills, <muscle, <kidney<liver in the winter and summer. The bioaccumulation order of Cu as kidney>liver>gills>muscle and gills>kidney>liver>muscle in winter and summer, respectively. The level of heavy metals was above the WHO-recommended safe limits. On histological evaluation, the kidney showed necrosis, bowman capsule space enlargement, and congestion. Cytoplasmic vacuolation, necrosis, and sinusoid dilatation were visible in the fish's liver.It has been determined that the effluent discharge of industrial waste and sewage water from the city has severely polluted this river and that urgent action is required to stop this pollution from spreading.

Keywords: heavy metals, fish, gills, muscle, effect.

P-62 Control and prevention of infectious disease of fish

Bharti Gupta and Ramakant Maurya
Department of Zoology, School of Science, Maharishi University of Information Technology Lucknow (India)
bg6568053@gmail.com

A fast expanding sector of agriculture worldwide is aquaculture. About 44% of the world's total fish production comes from it. Despite encountering numerous obstacles in the aquaculture environment, this significant rise of production is achieved. Infectious disease is the main factor restricting output since it results in yearly losses of several billions of dollars. It is vital to address health issues based on methods that have been scientifically verified and advised in order to lessen the impact of the fish disease. This review aims to highlight some of the most effective strategies for infectious disease prevention and control in aquaculture. Vaccination is one of the most important procedures among the efficient prevention and control techniques. Fish vaccines can come in a variety of forms, including DNA vaccines, recombinant technology vaccines, killed vaccinations, attenuated vaccines, and synthetic peptide vaccines. Fish vaccinations can be administered orally, intravenously, or subaerobically.Despite the negative effects of antibiotics on the emergence of drug resistance in microorganisms, they are nonetheless used in aquaculture. There is widespread usage of biological and chemical disease control methods, including the use of probiotics, prebiotics, and medicinal plants. Aquaculture biosecurity techniques can protect a facility against specific disease-causing agents that are not present in the system. Strict quarantine procedures, egg disinfection, traffic control, water treatment, clean feed, and mortalities disposal are all examples of farm-level biosecurity measures. In conclusion, it is advised to take a preventive approach before any disease outbreaks occur rather than attempting to treat every sickness case.

Certificate No. : CPP/ 47/360 /IEEC BHU-2023

ieec BHU
JANUARY 27-30, 2023

काशी हिन्दू विश्वविद्यालय
BANARAS HINDU UNIVERSITY

CERTIFICATE

OF PAPER PRESENTATION

This is to certify that Mr./Ms./Dr. BHARTI GUPTA
of Maharshi University of Info. Technology, Lucknow
has **Presented a Paper/Poster** entitled Sarayu River heavy metal poisoning on the histopathological alterations in various organs of channa punctatus
in the **International Extension Education Conference (IEEC BHU-2023)** on **Innovative Applications in Agricultural Extension for Sustainable Food & Environmental Security** organized by **Department of Agricultural Extension & Communication**, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (UP), India during **January 27-30, 2023.**

Prof. Kalyan Ghadei
Organizing Secretary
IEEC-BHU 2023
Head, Deptt. of Agricultural Extension & Communication
Institute of Agricultural Sciences, BHU, Varanasi

Prof. Yashwant Singh
Director, IEEC-BHU 2023
Director
Institute of Agricultural Sciences
Banaras Hindu University, Varanasi

>

NATIONAL CONFERENCE

Current Trends in Biological Sciences for Sustainable Agriculture Environment & Health Under Climate Change

XV Convention of the Indian Society of Agricultural Biochemists

NOVEMBER 23-25, 2023

Organized by

Indian Society of Agricultural Biochemists, CSAUA&T, Kanpur

&

Department of Botany, University of Lucknow, Lucknow

CERTIFICATE

This is to certify that Dr/Mr/Ms..... Bharti Gupta .....

participated/delivered ORAL/POSTER presentation in the National Conference and the XV convention of the ISAB, held at the University of Lucknow, Lucknow.

Prof. Munna Singh
Organizing Secretary

Prof. G.P. Srivastava
Hon. Gen. Secretary, ISAB

Online ISSN: 2588-3526
Journal of Wildlife and Biodiversity

Volume 8(2): 103-121 (2024) (http://www.wildlife-biodiversity.com/)

Review Article

Metalliferous uptake and Deposition in Fish in contaminated environments

Bharti Gupta, Ramakant Maurya*

Department of Zoology, School of Science, Maharshi University of Information Technology, Lucknow, India

*Email: bg6568053@gmail.com

Received: 25 January 2024 / Revised: 10 April 2024 / Accepted: 11 April 2024/ Published online: 13 April 2024.

How to cite: Gupta, B., Maurya, R. (2024). Metalliferous uptake and Deposition in Fish in contaminated environments, Journal of Wildlife and Biodiversity, 8(2), 103-121. **DOI**: https://doi.org/10.5281/zenodo.

Abstract

There is an accumulation of toxic metal ions in an aquatic habitat that modifies the physical and chemical characteristics of water, posing a threat to aquatic organisms. The fish's body absorbs heavy metals through the gills, dorsal surface, and gastrointestinal system when they eat foodstuff that has high levels of these metals. Zinc, Lead, Mercury, Copper, Arsenic, Nickel, chromium and cadmium are the primary heavy metal contaminants responsible for inducing toxicity in fish. Oxidative stress, or oxidative damage, is the primary chemical process responsible for metal poisoning. Stress undermines a low immune system, leading to harm to organs and tissues, developmental irregularities, and reduced reproductive capacity. Due to the copious availability of vitamins, proteins, and fatty acids such as omega-3 found in fish, individuals are inclined to consume seafood as their primary nutritional source. Consequently, the aggregation of toxic metallic elements in fish tissues has a direct impact on people, causing detrimental effects that accelerate the onset of various diseases. To effectively enforce aquatic conservation regulations and protect human lives, it is imperative to investigate the origins of toxic metals and their detrimental effects on the health of fish.

Keywords: Fish, copper, heavy metals, diet, rivers, Gomati, toxic metals

Introduction

The existence of elevated concentrations of toxic metals in water bodies presents a substantial peril to the organisms residing in these habitats, specifically fish (Elumalai *et al.* 2023). Heavy metals are inherent in the surroundings, but their overuse in various industries for varied reasons has greatly disrupted the ecological system (Sharma *et al.* 2023). This disruption is caused by the

excessive release of these elements into the soil and water bodies. Typically, human activities, such as growing crops, erosion from farms, and the release of industrial and residential trash, are (Kakade *et al.* 2023) recognized as the primary contributors to heavy metals in water systems (Saidon *et al.* 2024a). Upon entering aquatic systems, heavy metals undergo dissolution in water and readily accumulate among various components of aquatic species, such as fish. Consequently, these fish, which are tainted, act as a means of heavy metal exposure for consumers. (Chatha *et al.* 2023; Ozuni *et al.* 2010a). The buildup of heavy metals in fish through the process of bioaccumulation leads to various difficulties in terms of fish health and their physiological roles (Ozuni *et al.* 2010a). The degree of metal poisoning (cancer-causing, mutagenic, and teratogenic) varies greatly depending on the type of fish, the metal concentration, and the exposure period (Zhang *et al.* 2024) Water-dwelling species, such as fish, can get polluted with heavy metals that originate from both the water and sediments of aquatic environments (Nyarko *et al.* 2023).

Environmental contamination by metallic substances has a detrimental effect on the neurological system of fish, leading to a disruption in their ability to interact with their surroundings (Javanshir Khoei, 2023) The unregulated utilization and buildup of these metals have emerged as a significant health problem, as the majority of them cannot decompose into harmless forms. Consequently, they have detrimental impacts on human health and aquatic creatures (Ozuni *et al.* 2010b).

The existence of elevated concentrations of toxic metals in the natural world has a detrimental impact on fish's development and functions related to reproduction. This is evident through a decrease in their gonad somatic index (GSI), fertilization, fecundity, and rate of hatching (Green &Planchart, 2018). In addition, the presence of heavy metals hinders the proper development and advancement of embryos of fish as well as larvae. While certain metals are necessary for the functioning of living beings (Shahjahan *et al.* 2022a), the majority of them pose significant risks, even in minuscule quantities. In addition, certain metals like cadmium (Cd), arsenic (As), copper (Cu), chromium (Cr), lead (Pb), mercury (Hg), zinc (Zn), nickel (Ni), selenium (Se), and others, are not only extremely poisonous but also cause cancer and genetic mutations (Taslima *et al.* 2022). While various physic-chemical procedures can be used to eliminate harmful heavy metals, many of these strategies prove to be inefficient when the levels of metals are below 100 mg/L (Paschoalini & Bazzoli, 2021a). Due to the solubility of numerous heavy metals in water and their ability to dissolve in polluted water, separating them using physical methods is extremely challenging (Djedjibegovic *et al.* 2020). Bioremediation, which is a biological approach, can be a

favorable alternative to restore the natural state of the environment affected by heavy metal pollution (Shahjahan *et al.* 2022b). Bioremediation is widely recognized as an ecologically sound and sustainable method for mitigating various forms of water pollution, hence enhancing the efficiency of aquaculture systems (Garai *et al.* 2021a).

In general, bioremediation is highly efficient at decreasing the toxicity of contaminants by transforming them into less hazardous forms using either microorganisms (Paschoalini & Bazzoli, 2021b) or their enzymes, hence reducing contamination (Garai *et al.* 2021b). This approach is regarded as an environmentally beneficial and economically efficient technique for rejuvenating the polluted ecosystem (Moiseenko & Gashkina, 2020). Microorganisms possessing catabolic capabilities or their byproducts, such as enzymes and physiological surfactants, offer a novel approach to enhance the effectiveness of remediation (Saidon *et al.* 2024b).

Microorganisms can produce metals, which is commonly employed as an environmentally friendly method for mitigating metal-related pollution (Saidon *et al.* 2024c). The utilization of various microbes for the production of nanomaterials has been extensively utilized for wastewater treatment on a global scale (Rajak *et al.* 2024). The microorganism-synthesized nanoparticles can efficiently eliminate and reuse heavy metals from aquatic systems that are contaminated with heavy metals while maintaining their stability (Mehnaz *et al.* 2023a).

Multiple studies have documented that genetically modified microbes can effectively boost their ability to adsorb substances and be successfully utilized in the process of remediation (Mehnaz *et al.* 2023b). The ability of microorganisms to remediate can be improved by implementing various modifications, such as the addition of biochar, biosurfactants, compost, and inorganic fertilizers. In addition, various contemporary methods in microbe-assisted biological technologies, such as rhizoremediation, organisms with genetic modification (Saidon *et al.* 2024a), and nanotechnological assistance in microbial bioremediation, have been extensively utilized for the removal of various toxic heavy metals from the environment. There is currently a lack of complete information regarding the remediation of toxic heavy metals in fish, despite the detrimental effects caused by the buildup of these metals. Hence, this review provides a concise overview of the most up-to-date knowledge on the accumulation of heavy metals in fish and the advancements made in bioremediation methods.(Mehnaz *et al.* 2023b)

Deposition of Metals in Various Fish tissue

Bioaccumulation evaluation is a crucial indicator for monitoring the biogeochemical cycle of pollutants in the aquatic ecosystem. The harmful consequences and oxidation of heavy metals differ depending on their distinct forms and types of metal. Chromium (Cr) is often found in six different oxidation states (+1 to +6), with hexavalent Cr being particularly harmful to fish. Figure 1 demonstrates that fish in aquatic systems contaminated with heavy metals pose a significant danger, as they store these metals in several vital tissues such as the gills, the kidneys, and the liver. Fish need additional energy to adapt to this stressful state, which they obtain from stored resources such as protein, lipids, and carbs. Certain metals (such as Arsenic, Cadmium, Chromium, Copper, Iron, Mercury, Nickel, Lead, and Zinc) possess redox potential and have the ability to generate reactive oxygen species (ROS). These ROS are crucial for maintaining specific physiological processes in fish. Reactive oxygen species (ROS) serve as markers of oxidative stress, which impairs cellular function by breaking down proteins, lipids, and DNA. Heavy metals accumulate in various aquatic creatures in the food chain and pose significant health risks to humans when consuming polluted seafood. (Garai *et al.* 2021b).

The buildup of heavy metals in various fish organs is detailed in Table 1, while the diverse harmful impacts of heavy metals on fish are illustrated in Table 2.

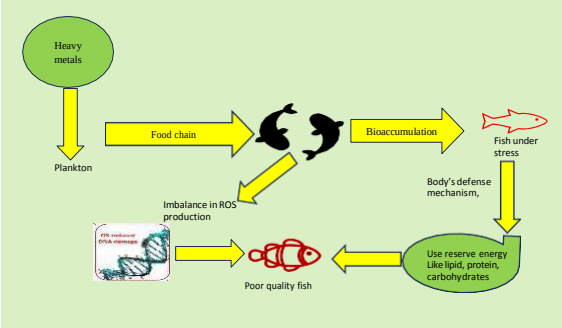

Figure 1. Routes of heavy metal buildup in fish include the presence of reactive oxygen species (ROS)

Table 1. Deposition of toxic metals in various fish tissues

Doses	Species	Exposure Time (Days)	Bioaccumulation Rate in Organs	References
Arsenic				
806.5 µg/g	*Oreochromis niloticus*	20	3.74 ± 3.38 µg/g in Muscle, 10.04 ± 2.99 µg/g in liver, 4.94 ± 4.62 µg/g in gills	(Garai *et al.* 2021a)

1500 µg/g	*Siganusfuscescens*	42	79.0–95.2% in muscle, 63.3–91.3% in liver	(Ozuni *et al.* 2010b)
Cadmium				
1.0 mg/L	*Oreochromis niloticus*	30	2.02–2.50 µg/g in muscle, 114.5–274.9 µg/g in liver, 22.34–32.26 µg/g in gills.	(Zhang *et al.* 2024)
5.03 mg/L	*Oreochromis niloticus*	30	0.08–1.41 µg/g in muscle, 0.41–138.12 µg/g in liver, 0.28 µg/g in gills	(Chatha *et al.* 2023)
5 mg/L	*Cyprinus carpio*	32	4.31–5.32 µg/g in the kidney, 4.82–5.64 µg/g in the liver, 6.23–6.94 µg/g in gills	(Sharma *et al.* 2023)
1.0 mg/L	*Oreochromis niloticus*	30	2.50 µg/g in muscle, 274.9 µg/g in liver, 32.26 µg/g in gills.	(Elumalai *et al.* 2023)
1500 mg/kg	*Oncorhynchus mykiss*	30	1.03–1.82 µg/g in Carcass, 1.20–6.47 µg/g in liver, 0.54–1.77 µg/g in gills.	(Saidon *et al.* 2024a)
Chromium				
3.41 mg/L	*Cyprinus carpio*	4	0.60–0.60 µg/g in bones, 0.30–0.35 µg/g in skin, 0.40–0.45 µg/g in muscle.	(Mehnaz *et al.* 2023b)
4.00 mg/L	*Cyprinus carpio*	1	3.21 µg/g in the skin, 3.9 µg/g in the intestine, 5.43 µg/g in the gills.	(Saidon *et al.* 2024d)
6.00 mg/L	*Cyprinus carpio*	2	3.03 µg/g in the skin, 3.63 µg/g in the intestine, and 4.69 µg/g in the gills.	(Ferdous *et al.* 2024)
Copper				
5.0 mg/L	*Oreochromis sp.*	ND	1.4–4.0 mg/kg in muscle 19.4–136 mg/kg in liver, 6.3–38.4 mg/kg in gills.	(Hamada *et al.* 2024)
0.1 mg/L	*Sparus aurata*	11	0.85–1.49 µg/g in muscle, 3.24–7.02 µg/g in liver.	(Hamada *et al.* 2024)

Table 2. Fish toxicity caused by heavy metals

Species	Toxicity	**References**
Arsenic		
Tilapia mossambica	The hemato-biochemical analysis revealed a large increase in the levels of white blood cells, MCHC, and MCH while the levels of Hb, red blood cells, and PCV showed a substantial decrease.	(Bemani&Okati, 2024)
Clarias batrachus	Hematological: There was a considerable decrease in serum protein level.	(Sevak & Pushkar, 2024)

Danio rerio	Reproduction: The quantity of the eggs, and hatching rate experienced a substantial decrease.	(Misra *et al.* 2024)
Clarias batrachus	Kidney: Contains vacuoles and melanomacrophages.	(Misra *et al.* 2024)
Oreochromis mossambicus	Liver: Presence of infiltrated macrophages as the reduction in size and congestion of hepatic cells, enlargement, and formation of vacuoles	(Sevak & Pushkar, 2024)
Cadmium		
Channa striata	The hemato-biochemical analysis revealed a rise in HDL, TP, as well as ALT levels, but the Glu level fell.	(Panigrahi *et al.* 2024)
Clarias gariepinus	The hemato-biochemical analysis revealed an increase in AST, ALT, Glu, as well as MCH levels, while CK and MCV levels were found to be lowered.	(Panigrahi *et al.* 2024)
Pelteobagrusfulvidraco	Markedly reduced weight gain as well as particular growth rate	(Bautista *et al.* 2024)
Chromium		
Anabas testudineus	The renal tissues exhibited the presence of kidney edema, interstitial hemorrhage, and deteriorated renal tubules.	(Kafouris *et al.* 2024)
Pangasianodonhypophthalmus	Erythrocyte abnormalities: Various aberrations detected in blood cells The levels of red blood cells (RBC), hemoglobin (Hb), and packed cell volume (PCV) showed a considerable drop in the field of hematology.	(Kafouris *et al.* 2024)
Oreochromis niloticus	There was a decrease in both weight gain as well a particular growth rate.	(Kafouris *et al.* 2024)
Oryziasmelastigma	The liver exhibits the presence of vacuoles pyknotic cells, and aberrant nuclei in its hepatic cells.	(Bautista *et al.* 2024)
Copper		

Leuciscus idus	Malformation of the yolk sac decreased lower-extremity length and the perimeter, and curved vertebrae.	(Yasmeen & Rafique, 2024)
Oryziasmelastigma	Malformed skeletal features, abnormalities in the circulatory system, reduced pigmentation of the embryos	(Chatha *et al.* 2024)
Poecilia reticulata	Reproduction: Substandard reproductive outcomes, prolonged birthing duration, elevated larval mortality	(Chatha *et al.* 2024)

Different heavy metals' harmful effects and bioaccumulation

Chromium

Both seawater and the Earth's crust contain minute quantities of chromium. This element is not present solely as a metal, but can be found in the environment in three different oxidation states: divalent ($Cr^{2+),}$ trivalent ($Cr^{3+),}$ and hexavalent (Cr^{6+}). The most stable variants among these are Cr^{3+} and Cr^{6+}. The Cr^{3+} oxidation state poses less risk due to its limited membrane permeability, non-corrosive properties, and reduced potential for bioaccumulation in the food chain. Cr^{6+} poses a greater risk because of its robust ability to cause oxidation and its tendency to infiltrate cell membranes. (Prabakaran *et al.* 2024)

Anthropogenic sources such as leather tanneries, metal manufacturing, petroleum refining, textile production, alloy preparation, and wood preservation contribute to the presence of chromium toxicity in aquatic ecosystems. The toxicity of chromium to aquatic creatures is affected by several biotic factors such as age, developmental stage, and species type, as well as abiotic variables including pH, temperature, and alkalinity of the water. The fish that were initially exposed to chromium exhibited a variety of behavioral abnormalities, such as irregular swimming patterns, excessive mucus production, alterations in body pigmentation, decreased hunger, and other symptoms(Chatha *et al.* 2024). *Cyprinus carpio* exhibited cytotoxicity, decreased lymphocyte activation in response to mitogens, and modified phagocyte activity following prolonged exposure to chromium at concentrations ranging from 2 to 200 mol/L. The presence of chromium in *Tilapia sparrmanii* resulted in the observation of internal hemorrhaging, as evidenced by a decreased blood coagulation time and an elevated pH level. Chromium accumulation in the tissues of the Indian big carp leads to a reduction in protein and fat content in the muscle, liver, and gill. Before

chromium therapy, *Colisa fasciatus*, a freshwater teleost, exhibited depleted levels of liver glycogen. Exposure to Cr^{6+} at pH levels of 7.8 and 6.5 caused respiratory and osmoregulatory failure in rainbow trout, *Salmo gairdneri*. Chronic chromium exposure had several detrimental consequences on Chinook salmon, such as DNA damage, microscopic lesions, physiological anomalies, and reduced growth and survival rates. The hatching of rainbow trout *Salmo gairdneri* embryos and the growth of the fish were impacted by the presence of chromium at a concentration of 2 mg/L. The chromium concentration in fish tissues differs, as seen in Table 1. The gills, liver, and kidney contain the highest levels of chromium, whereas the lowest levels are present in muscle tissue. (Chatha *et al.* 2024).

Cadmium (Cd)

Cadmium is a trace element found in the earth's crust at concentrations ranging from 0.1 to 0.5 parts per million. It is commonly present in zinc, copper, and lead ores. The mean content in ocean water varies between 5-110 mg/L, but surface and groundwater generally exhibit amounts below 1 ug/L. Naturally occurring cadmium does not exist in elemental form. Instead, cadmium chloride, cadmium oxide, cadmium sulfide, cadmium carbonate, cadmium nitrate, and cadmium cyanide are frequently seen. Cadmium enters marine environments through several natural and anthropogenic sources (Bautista *et al.* 2024). Cadmium is extracted from the earth's crust and mantle through volcanic eruptions and the process of rock weathering. Researchers have identified several anthropogenic sources of pollution, which include the combustion of fossil fuels, the use of fertilizers, the disposal of agricultural waste, and the industrial utilization of plastic stabilizers, pigments, batteries, and electroporation. Cadmium, a non-essential metal, poses a significant threat to fish due to its high level of toxicity. The study by researchers demonstrated that it enhances the generation of reactive oxygen species (ROS) while inhibiting the electron transport chain in mitochondria. *Cyprinus carpio* had DNA damage as a result of little cadmium exposure (Bautista *et al.* 2024). Cadmium ions (Cd^{+2}) were discovered to limit the movement of calcium across the epithelial cells in the gills of rainbow trout. Exposure to cadmium chloride for a relatively short period caused fish to generate cells with abnormal nuclei in their blood, gills, and liver. Fish that were subjected to cadmium had a distinct hematological reaction. The kidney tissue of tilapia exhibited histological alterations including hepatic fatty vacuolation, hepatocyte necrosis,

submucosal blood vessel congestion in the gut, and glomerular shrinkage and necrosis. Exposure of American eel fish *(Anguilla rostrata*) to a concentration of 150 g/L of cadmium for 8 weeks leads to the development of anemia, characterized by a drop in both hemoglobin and erythrocyte counts. Following exposure to cadmium, there was a notable increase in the number of leukocytes and large lymphocytes. Exposure of *Cyprinus carpio* to sublethal concentrations of cadmium resulted in a significant decrease in glycogen reserves in both the muscle and liver, accompanied by an increase in blood glucose levels. Cadmium, a known endocrine disruptor, has been discovered in rainbow trout, Oncorhynchus mykiss, where it inhibits vitellogenesis (Panigrahi *et al.* 2024) Exposure to cadmium chloride had an impact on both the gonad function and sexual maturity of the common carp *Cyprinus carpio*. The larvae of *Leuciscus idus* that were exposed to cadmium had physical deformities and a reduced rate of embryonic survival as a result of mortality in freshly emerged larvae. The slow excretion rate of cadmium leads to significant environmental risks due to its accumulation. The epidermis exhibits the least amount of cadmium bioaccumulation, while the liver, kidney, and gills demonstrate the highest levels. The gill is the organ that efficiently eliminates cadmium toxins. Cadmium poses a significant threat to aquatic organisms due to its high bioaccumulation rate, making it one of the most dangerous heavy metals(Kafouris *et al.* 2024).

Lead (Pb):

Lead, when mixed with components like oxygen and nitrogen, is considered one of the most hazardous heavy metals in existence. Various human activities, such as metal mining, combustion of coal, oil, and gasoline, battery manufacturing, use of lead-arsenate pesticides, lead-based paint, pigments, and food cans, significantly elevate the levels of lead in the environment (in the form of PbS, $PbSO_4$, and $PbCO_3$). The aquatic environment is promptly affected by the release of lead from various industries, agricultural fields, street runoff, lead dust, and municipal wastewater, resulting in toxicity to aquatic organisms. The solubility of lead in water is influenced by several elements such as pH, salinity, hardness, and other variables. Lead quickly dissolves in water that is both soft and acidic. The acceptable range for lead exposure in fish is limited to concentrations ranging from 10 to 100 mg/L (Kafouris *et al.* 2024). Sublethal lead exposure causes fish to have modified behavior, reproductive dysfunction, and stunted growth. Katti observed that prolonged exposure to a small amount of lead nitrate resulted in alterations in the lipid and cholesterol

composition of the liver, brain, and gonads of *Clariasbatrachus*(Panigrahi *et al.* 2024). Lead exposure resulted in histological abnormalities in the gill and liver tissue *of Clarias gariepinus*, an African catfish species. Exposure to lead caused histological alterations in the ovarian tissue of freshwater teleosts (*Mastacembeluspancalus*). Lead-exposed fish exhibited symptoms of parenchyma cell necrosis, hepatic cord, and connective tissue fibrosis, reduced growth and body weight, and blood vessel collapse. *Nile tilapia* exposed to lead exhibited decreased levels of hemoglobin, red blood cell count, and hematocrit values. Lead exposure induces oxidative stress in fish, leading to synaptic damage and neurotransmitter dysfunction. Exposure to lead, both in lethal and non-lethal doses, caused alterations to the immunological parameters of Tench fish (*Tinca tinca*). Researchers have identified the liver, spleen, kidney, and gills as the main locations where lead bioaccumulates in fish. Lead bioaccumulation caused morphological defects in Acipenser sinensis, a Chinese sturgeon species.(Panigrahi *et al.* 2024).

Impact of heavy metals on reproductive hormones

Hormones related to reproduction have a crucial role in the effective breeding of fish. Gonadotropin (GTH) is a crucial hormone in the control of fertility. GTH serves two separate functions in terms of structure and chemistry and exists in two distinct forms. GTH-I is responsible for spermatogenesis, which is the early stage of gametogenesis. On the other hand, GTH-II participates in sperm formation and spermiation. The hypothalamus secretes GnRH, a hormone that stimulates the pituitary gland to produce and synthesize GTH, specifically FSH and LH. FSH and LH control the yearly reproductive cycle, including the generation of sex hormones in both males and females, fertilization in females, and the release of sperm in males. FSH and LH can stimulate the production of steroids and the development of gametes in the gonads.(*Gautam et al.* 2024)

Gonadotropic hormones are transported to the gonads, where they stimulate steroidogenesis, leading to the synthesis of sex steroid hormones that control the reproductive process. These hormones also interfere with the regulatory functions of the pituitary gland and hypothalamus through mechanisms of feedback. Gonadotropin release interruption can profoundly affect fertility. The synthesis of fish hormones for reproduction is disrupted when there is a compromise in the hypothalamic-pituitary system. Testosterone, a sexual hormone, plays a role in the growth of gonads at the end of the period of menstruation and serves as a precursor for the generation of estradiol.(Echevarría *et al.* 2024)

Women are the primary synthesizers of estradiol (E2). Additionally, it plays a vital function in men by controlling the growth of spermatogonia and the functioning of Sertoli cells, both of these are controlled by nuclear estrogen receptors (ERs). Estrogen receptors (ERs) play a crucial role in the differentiation of sexuality and maturation processes, including the development of testes, oogenesis, as well as vitellogenesis. The reference is from a study conducted by Kim *et al.* in 2014. Estradiol is a hormone that is required for the stimulation of calcitonin production, which in turn affects the secretion of vitellogenin and the development of oocytes. Testosterone levels increase as the gonads develop in both male and female fish. The growth of males' testicles is regulated by the hormones testosterone (T) and ketotestosterone (11-KT), with 11-KT being more potent than T(Marinaro *et al.* 2024). 11-KT is believed to play a significant role in male sexual behavior and the release of LH. This mechanism involves an intricate combination of endocrine and neuroendocrine input from many receptors, together with local autocrine as well as paracrine secretory modulation and feedback control. Contaminants that disturb the balance of gonadotropin and sexual hormones can potentially affect the reproductive process of fish.Sex hormonal substances are crucial in the processes of sex differentiation, maturity, and reproduction (Gautam *et al.* 2024; Marinaro *et al.* 2024)

The decline in hormone levels caused by pollution can be used as important indicators and tools to evaluate the impact of stress on fish. Cadmium is a highly dangerous metal for fish, as it disrupts the endocrine system and has various negative effects on reproduction. These effects include changes in additional sexual traits, increased levels of 11-ketotestosterone, a decrease in the gonadosomatic index, reduced sperm motility, and alterations in estrogen levels in Nile Tilapia due to exposure to Cadmium. Garriz et al. (2017) discovered that male *pejerrey* fish who received Cd had elevated levels of testosterone-releasing hormone expression. Cadmium (Cd) may inhibit the correct functioning of enzymes involved in the production of sex hormones in the gonads and the metabolism of steroids in the liver. Exposure to cadmium concentrations of 0.4 ppm or 4 ppm has been shown to result in an elevation of LH levels in the blood plasma of *Carassius gibelio B*, together with a delay in gonad maturity.(Zulfahmi *et al.* 2024).

Out of all heavy metals, only a few number, including cadmium, zinc, copper, lead, and mercury, have been discovered to have detrimental effects. Consequently, the majority of studies have concentrated on investigating the implications of these specific heavy metals. Approximately 33% of the Cadmium present in aquatic habitats is believed to originate from the production and use of

phosphate fertilizers. The exposure of fish to Cd can lead to the buildup of the substance, with the specific processes varying according to the trophic level of the species. This particular heavy metal exerts a distinctive influence on sperm cells. The discussion focused on the effects of the substance on the quality of sperm in several species of freshwater fish, such as *D. rerio* , *C. carpio* , and *Gymnotuscarapo* . Treatment with Cadmium (Cd) also led to decreased motility and velocities in the spermatozoa of *Prochilodusmagdalenae*. The study conducted by (Vasconcelos *et al.* 2024) revealed that exposing *Colossomamacropomum* to cadmium concentrations of 0, 0.6, 1.2, and 1.8 ppm resulted in a decrease in the motility rate of spermatozoa. Furthermore, the rates of fertilization and hatching were also shown to be reduced. The exposure of *Prochilodusmagdalenae* to cadmium at concentrations of 2.5 parts per million (ppm) and 25 ppm for 27.3 seconds resulted in a decrease in both sperm motility and velocity, as reported by (Zulfahmi *et al.* 2024). In addition, the study conducted by (Gautam *et al.* 2024) revealed a decrease in the quality of eggs and sperm in Gasterosteus aculeatus when exposed to a level of 1ppb of cadmium for durations of 15, 60, and 120 days. The study conducted by (Bautista *et al.* 2024) discovered that exposure to cadmium at concentrations ranging from 20 to 110 ppm in Rhamda queen resulted in decreased sperm motility. The provided information corresponds to Table 3.

Table 3. Impact of metal poisoning on fish reproductive processes

Heavy metals	Fish name	Effects	References
Cd	*Trematomusbernacchii*	Oocyte deterioration leads to a decrease in fertility.	(Bautista *et al.* 2024)
Cd	*Colossomamacropomum*	The decline in the rate of fertilization and hatching	(Kafouris *et al.* 2024)
Cd	*Oreochromis spp.*	Suppressed reproductive process in females, compromised growth of ovaries, a notable decline	(Kafouris *et al.* 2024)
Pb	*Oreochromis niloticus*	The Nile tilapia larvae exhibit deformities such as lordosis, kyphosis, and bent tails.	(Waichman *et al.* 2024)
Cu	*Trematomusbernacchii*	Oocyte degeneration leads to a decrease in fertility. The motility rate of spermatozoids has decreased, resulting in a fall in both fertilization and hatching rates.	(Waichman *et al.* 2024)
Cu	*Danio rerio*	The reduction of gonad maturation is associated with a decrease in gonadsomatic index (GSI) and steroidogenesis.	(Waichman *et al.* 2024)

Cu	*P. vivipara*	Disruption of spermatic mitochondria and neuroendocrine functioning	(Vasconcelos *et al.* 2024)
Cu	*Gambusia affine*	Premature births	(Vasconcelos *et al.* 2024)
Cu	*Pelteobagrusfulvidraco*	Egg yolk granules, ovarian metamorphosis,Oncorhynchus mykiss Modified mitochondria	(Vasconcelos *et al.* 2024)

Remediation of Heavy Metal Toxicity in Fish via Bioremediation

Bioremediation is a practical and environmentally beneficial approach that can be employed to remediate a polluted environment by extracting harmful metals from it (Rengarajan *et al.* 2024). Bioremediation of toxicants can be achieved through adsorption, physio-biochemical pathways and molecular processes. catalase (CAT), Superoxide dismutase (SOD), reduced glutathione (GSH), and glutathione S transferase (GST), are important in maintaining the balance of reactive oxygen species (ROS) through detoxification (Figure 2). SOD possesses the ability to transform superoxide radicals into hydrogen peroxide radicals, which subsequently transform into harmless oxygen and water radicals through the action of CAT enzymes (Rengarajan *et al.* 2024). Conversely, GST facilitates the detoxification of harmful substances by catalyzing the conversion of electrophiles into GSH. In addition, GSH undergoes nonenzymatic oxidation of electrophilic molecules, such as free radicals and ROS, resulting in the conversion of GSH into glutathione disulfide (Kurella *et al.* 2024a).Microorganisms possess many mechanisms that confer resistance to heavy metals, such as extracellular sequestration, intracellular sequestration, reduction of heavy metal ions within the microbial cell, and the presence of extracellular barriers. Various microorganisms, including bacteria, fungi, and algal species, have been employed to eliminate toxic heavy metals and maintain environmental cleanliness (Ozturk *et al.* 2024). These microorganisms are included in Table 3. Furthermore, alongside natural microbes, several genetically enhanced artificial microorganisms, particularly those that have been modified on their surface, have been created to utilize in the process of remediating specific heavy metals. Multiple studies have documented that genetically modified microorganisms possess superior skills compared to normal bacteria in eliminating organic substances, such as heavy metals, within natural environmental systems (Khan, 2024)

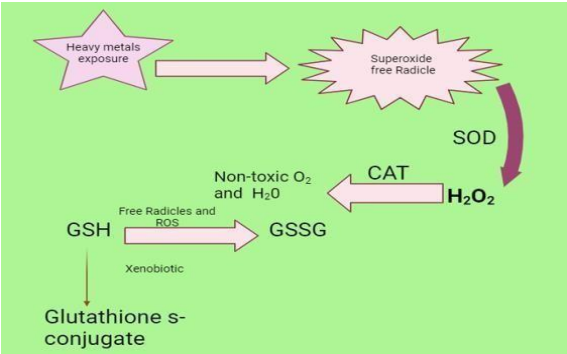

Figure 2. The mechanism of detoxification of heavy metals involves the enzymes SOD, CAT, and GST, as well as the molecules GSH, GSSG, and ROS.

Bio precipitation

The bioavailability and toxicity of hazardous metals can be decreased when they precipitate into insoluble complexes. Many microorganisms are reported to facilitate the bio-precipitation of heavy metals. For instance, *Providentiaalcalifaciens* 2EA, a lead-resistant strain bio precipitates Pb as lead phosphate i.e. $Pb_9(PO_4)_6$. Lead has also been found to precipitate as PbSby *Klebsiella sp.* grown on phosphate-limited media.Both lead-sensitive and lead-resistant strains of *S. aureus* were found capable of precipitating lead, but the resistant variants were more successful. (Ren *et al.* 2024). *Pseudomonas sp.* was observed to produce an insoluble compound that contained both lead and phosphorus, indicating that the substance was lead phosphate. Lead is also precipitated by lead-resistant strain *Bacillus iodinium GP13 and Bacillus pumilus S3* as lead sulphide (PbS) (Khan, 2024). *E. cloacae,* which is phosphate solubilizing bacteria, resists lead by immobilizing it as the insoluble lead phosphate mineral pyromorphite. The microbial precipitation approach has proven to be an efficient, reasonably economical, and environmentally acceptable technical alternative for the reclamation of lead-polluted surroundings (Ren *et al.* 2024)

According to one study by (Agarwal *et al.* 2024). *W6* bacteria adsorbed 66% Pb from synthetic groundwater in Bangladesh at the ideal pH and temperature, compared to *P. Aeruginosa MTCC 2474* bacteria (19.9%), *P. alcaligenes MJ7* bacteria (45.3%), and *P. ficuserectae PKRS 11* bacteria (29.8%). *Pseudomonas sp. W6's* ability to remove lead from synthetic water demonstrated that it can also promote Pb remediation in natural water.

(Mangueina *et al.* 2024; Ren *et al.* 2024) most recent study found that the microorganisms *Spirogyra spp.* and *Cladophora spp.*could effectively sequester lead with oxidation state II, or Pb (II) as well as Cu (II). Similarly, *Spirogyra* and *Spirulina* species can sequester a variety of heavy

metals, including Cr, Cu, Fe, Mn, and Zn. Despite its increased scope, studies, as well as practical applications, are advised since the blending of different tactics could demonstrate the potential for the recovery of contaminated environments. Given below is a depiction of some potential microorganisms that are utilized to sequester certain heavy metals. (Table 4).

Table 4. Sequestration of different heavy metals by microorganisms.

Class of Microorganisms	Heavy Metal Sequestered	References
1. Bacteria		
Bacillus cereus	Cr (VI)	(Ren *et al.* 2024)
Kocuria flava	Cu	(Ren *et al.* 2024)
Sporosarcinaginsengisoli	As (III)	(Ren *et al.* 2024)
Bacillus cereus strain XMCr-6	Cr (VI)	(Khan, 2024)
Pseudomonas veronii	Cd, Zn, Cu	(Ozturk *et al.* 2024)
Enterobacter cloacae B2-DHA	Cr (VI)	(Khan, 2024)
Pseudomonas putida	Cr (VI)	(Kurella *et al.* 2024b)
Bacillus subtilis	Cr (VI)	(Kurella *et al.* 2024b)
2. Fungi		
Aspergillus fumigatus	Pb	(Rengarajan *et al.* 2024)
Gloeophyllumsepiarium	Cr (VI)	
Aspergillus versicolor	Ni, Cu	
Rhizopus oryzae (MPRO)	Cr (VI)	
3. Yeast		
Sacharomyces cerevisiae	Pb, Cd	(Rengarajan *et al.* 2024)
4. Algae		
Hydrodictylon, *Oedogonium* and *Rhizoc lonium* spp.	As	(Rengarajan *et al.* 2024)
Spirogyra spp. and *Spirullina* spp.	Cr Cu, Fe, Mn, Zn	
Spirogyra spp. and *Cladophora* spp.	Pb (II), Cu (II)	

Conclusion

The uncontrolled release of hazardous heavy metals from many sectors is causing significant degradation of aquatic habitats. Consequently, dangerous heavy metals originating from this polluted environment have built up in several vital organs of fish and disrupted their regular processes. The buildup of these hazardous metals in the bodies of fish has significantly impacted their normal physiological functions, leading to decreased fish growth and reproduction. Bioremediation can effectively address and transform current contaminations in aquatic systems using a sustainable manner. In addition, bioremediation enhances fish well-being by modifying the detrimental impacts of several heavy metals. Not only does it provide benefits for aquatic creatures, but it also enhances the productivity of aquatic ecosystems. Through the effective implementation of this bioremediation process, we may greatly reuse the water, hence minimizing water wastage.

Additionally, the breakdown of organic materials in the water reduces the presence of harmful organisms, thus improving the overall biosecurity of our ecosystems. In addition to present bioremediation approaches, the future implementation of genetically engineered microorganisms (GEM) should be considered to enhance the effectiveness of bioremediation strategies in addressing harmful heavy metal pollution. It is important to evaluate the public acceptance of GEM and the environmental safety in this situation.

References:

Agarwal, S., Sharma, G., & Mathur, V. 2024. Plant Endophytes: A Treasure House of Antimicrobial Compounds. In *Medicinal Plants and Antimicrobial Therapies* (pp. 107–123). Springer.

Bautista, C. J., Arango, N., Consuelo, P., Mitre-Aguilar, I. B., Trujillo, J., & Ramírez, V. 2024. Mechanism of Cadmium-induced nephrotoxicity. *Toxicology*, 153726.

Bemani, A., &Okati, N. 2024. Risk Assessment of Some Heavy Metals (Lead, Chromium, Mercury, Arsenic, and Nickel) in Nile Tilapia (Oreochromis niloticus) Cultured in the Biofloc System. *Environment and Water Engineering*.

Chatha, A. M. M., Naz, S., Iqbal, S. S., Kiran, A., Lateef, M., Zahra, U., Yasmin, F., Amjad, N., & Javaid, A. 2024. Detection of DNA Damage in Fish using Comet Assay. *Current Trends in OMICS*, *4*(1), 1–16.

Chatha, A. M. M., Naz, S., Iqbal, S. S., & Zahra, U. 2023. Investigating the Impact of Environmental Toxicology of Heavy Metals in Fish: A Study of Rivers of Pakistan. *Scientific Inquiry and Review*, *7*(4), 67–90.

Djedjibegovic, J., Marjanovic, A., Tahirovic, D., Caklovica, K., Turalic, A., Lugusic, A., Omeragic, E., Sober, M., &Caklovica, F. 2020. Heavy metals in commercial fish and seafood products and risk assessment in adult population in Bosnia and Herzegovina. *Scientific Reports*, *10*(1), 13238.

Echevarría, G., Lujan, N. K., Montoya, J., Granda-Albuja, M. G., Valdiviezo-Rivera, J., Sánchez, F., Cuesta, F., & Ríos-Touma, B. 2024. Abiotic and biotic factors influencing heavy metals pollution in fisheries of the Western Amazon. *Science of The Total Environment*, *908*, 168506.

Elumalai, S., Prabhu, K., Selvan, G. P., & Ramasamy, P. 2023. Review on heavy metal contaminants in freshwater fish in South India: current situation and future perspective. *Environmental Science and Pollution Research*, 1–18.

Ferdous, Z., Rafiquzzaman, S. M., & Shahjahan, M. 2024. Probiotics ameliorate chromium-induced growth retardation and stress in Indian major carp rohu, Labeorohita. *Emerging Contaminants*, *10*(2), 100291.

Garai, P., Banerjee, P., Mondal, P., & Saha, N. C. 2021a. Effect of heavy metals on fishes: Toxicity and bioaccumulation. *J Clin Toxicol. S*, *18*.

Garai, P., Banerjee, P., Mondal, P., & Saha, N. C. 2021b. Effect of heavy metals on fishes: Toxicity and bioaccumulation. *J Clin Toxicol. S*, *18*.

Gautam, R., Priyadarshini, E., Patel, A. K., & Arora, T. 2024. Assessing the impact and mechanisms of environmental pollutants (heavy metals and pesticides) on the male reproductive system: a comprehensive review. *Journal of Environmental Science and Health, Part C*, 1–28.

Green, A. J., &Planchart, A. 2018. The neurological toxicity of heavy metals: A fish perspective. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *208*, 12–19.

Hamada, N. H. S., Ali, R. A. S., El-Tabakh, M. A. M., & Bream, A. S. 2024. Environmental monitoring: Tobara fish as bioindicators of heavy metal pollution in a coastal ecosystem. *Regional Studies in Marine Science*, *69*, 103335.

Javanshir Khoei, A. 2023. A comparative study on the accumulation of toxic heavy metals in fish of the Oman Sea: effects of fish size, spatial distribution and trophic level. *Toxin Reviews*, *42*(1), 189–196.

Kafouris, D., Christoforou, E., Stefani, D., Sarandi, A., Stavroulakis, G., Christou, E., & Yiannopoulos, S. 2024. Lead, Cadmium and Mercury Determination and Human Health Risk Assessment in Foods from Cyprus. *Journal of Food Composition and Analysis*, 106007.

Kakade, A., Sharma, M., Salama, E.-S., Zhang, P., Zhang, L., Xing, X., Yue, J., Song, Z., Nan, L., & Yujun, S. 2023. Heavy metals (HMs) pollution in the aquatic environment: Role of probiotics and gut microbiota in HMs remediation. *Environmental Research*, *223*, 115186.

Khan, M. S. A. 2024. Applications of Bioremediation in Biomedical Waste Management: Current and Future Prospects. *Brazilian Archives of Biology and Technology*, *67*, e24230161.

Kurella, B. R., Ramanujan, A. S., Nagaraj, B., Narayanappa, R., &Kunnel, S. G. 2024a. Chemically modified Ginger and Spirulina for bioremediation of Hexavalent Chromium from polluted water. *Journal of Integrated Science and Technology*, *12*(4), 782.

Kurella, B. R., Ramanujan, A. S., Nagaraj, B., Narayanappa, R., &Kunnel, S. G. 2024b. Chemically modified Ginger and Spirulina for bioremediation of Hexavalent Chromium from polluted water. *Journal of Integrated Science and Technology*, *12*(4), 782.

Mangueina, D., Awuah, E., Fonteh, M., Agyei, P. A., & Nadji, E. T. 2024. Assessment of current water, sanitation, and hygiene (WASH) practices in the third and ninth districts of N'Djamena, Chad. *Journal of Water and Health*, jwh2024324.

Marinaro, C., Lettieri, G., Chianese, T., Bianchi, A. R., Zarrelli, A., Palatucci, D., Scudiero, R., Rosati, L., De Maio, A., & Piscopo, M. 2024. Exploring the molecular and toxicological mechanism associated with interactions between heavy metals and the reproductive system of Mytilus galloprovincialis. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *275*, 109778.

Mehnaz, M., Jolly, Y. N., Alam, A. K. M. R., Kabir, J., Akter, S., Mamun, K. M., Rahman, A., & Islam, M. M. 2023a. Prediction of Hazardous Effect of Heavy Metals of Point-Source Wastewater on Fish (Anabas cobojius) and Human Health. *Biological Trace Element Research*, *201*(6), 3031–3049.

Mehnaz, M., Jolly, Y. N., Alam, A. K. M. R., Kabir, J., Akter, S., Mamun, K. M., Rahman, A., & Islam, M. M. 2023b. Prediction of Hazardous Effect of Heavy Metals of Point-Source Wastewater on Fish (Anabas cobojius) and Human Health. *Biological Trace Element Research*, *201*(6), 3031–3049.

Misra, A., Bissessur, A., Selala, M. C., & Lebepe, J. 2024. Accumulation and health implications of arsenic, mercury, and selenium in selected freshwater fish species in the uMgeni River, South Africa. *Environmental Pollutants and Bioavailability*, *36*(1), 2296973.

Moiseenko, T. I., &Gashkina, N. A. 2020. Distribution and bioaccumulation of heavy metals (Hg, Cd and Pb) in fish: Influence of the aquatic environment and climate. *Environmental Research Letters*, *15*(11), 115013.

Nyarko, E., Boateng, C. M., Asamoah, O., Edusei, M. O., & Mahu, E. 2023. Potential human health risks associated with ingestion of heavy metals through fish consumption in the Gulf of Guinea. *Toxicology Reports*, *10*, 117–123.

Ozturk, M., Unal, B. T., & Turker, H. 2024. Biotechnological Interventions for Removal of Heavy Metals and Metalloids from Water Resources. *Aquatic Contamination: Tolerance and Bioremediation*, 207–217.

Ozuni, E., Dhaskali, L., Abeshi, J., Zogaj, M., Haziri, I., Beqiraj, D., & Latifi, F. 2010a. Heavy metals in fish for public consumption and consumer protection. *Natura Montenegrina*, *9*(3), 843–851.

Ozuni, E., Dhaskali, L., Abeshi, J., Zogaj, M., Haziri, I., Beqiraj, D., & Latifi, F. 2010b. Heavy metals in fish for public consumption and consumer protection. *Natura Montenegrina*, *9*(3), 843–851.

Panigrahi, A. K., Pal, P. K., & Sarkar, D. 2024. Melatonin as an Ameliorative Agent Against Cadmium-and Lead-Induced Toxicity in Fish: an Overview. *Applied Biochemistry and Biotechnology*, 1–31.

Paschoalini, A. L., & Bazzoli, N. 2021a. Heavy metals affecting Neotropical freshwater fish: A review of the last 10 years of research. *Aquatic Toxicology*, *237*, 105906.

Paschoalini, A. L., & Bazzoli, N. 2021b. Heavy metals affecting Neotropical freshwater fish: A review of the last 10 years of research. *Aquatic Toxicology*, *237*, 105906.

Rajak, P., Ganguly, A., Nanda, S., Mandi, M., Ghanty, S., Das, K., Biswas, G., & Sarkar, S. 2024. Toxic contaminants and their impacts on aquatic ecology and habitats. In *Spatial Modeling of Environmental Pollution and Ecological Risk* (pp. 255–273). Elsevier.

Ren, J., Liu, B., Guo, J., Liu, J., Xing, F., Zhu, H., Zhao, L., & Mi, T. 2024. Bio-treatment of municipal solid waste incineration fly ash: A sustainable path for recyclability. *Journal of Cleaner Production*, *434*, 139869.

Rengarajan, S., Deepa, S., Natarajan, D., Pandian, A., Al-Ansari, M. M., Oza, G., Castillo-Maldonado, I., & Sharma, A. 2024. Bioremediation potential of biochar and metal tolerant Bacillus cereus on heavy metal polluted mine surrounding pond and assessed cytotoxicity and phytotoxicity attributes of treated water on Brine shrimp larvae and Paddy seedling. *Journal of the Taiwan Institute of Chemical Engineers*, 105330.

Saidon, N. B., Szabó, R., Budai, P., & Lehel, J. 2024a. Trophic transfer and biomagnification potential of environmental contaminants (heavy metals) in aquatic ecosystems. *Environmental Pollution*, *340*, 122815.

Saidon, N. B., Szabó, R., Budai, P., & Lehel, J. 2024b. Trophic transfer and biomagnification potential of environmental contaminants (heavy metals) in aquatic ecosystems. *Environmental Pollution*, *340*, 122815.

Saidon, N. B., Szabó, R., Budai, P., & Lehel, J. 2024c. Trophic transfer and biomagnification potential of environmental contaminants (heavy metals) in aquatic ecosystems. *Environmental Pollution*, *340*, 122815.

Saidon, N. B., Szabó, R., Budai, P., & Lehel, J. 2024d. Trophic transfer and biomagnification potential of environmental contaminants (heavy metals) in aquatic ecosystems. *Environmental Pollution*, *340*, 122815.

Sevak, P., & Pushkar, B. 2024. Arsenic pollution cycle, toxicity and sustainable remediation technologies: A comprehensive review and bibliometric analysis. *Journal of Environmental Management*, *349*, 119504.

Shahjahan, M., Taslima, K., Rahman, M. S., Al-Emran, M., Alam, S. I., &Faggio, C. 2022a. Effects of heavy metals on fish physiology–a review. *Chemosphere*, *300*, 134519.

Shahjahan, M., Taslima, K., Rahman, M. S., Al-Emran, M., Alam, S. I., &Faggio, C. 2022b. Effects of heavy metals on fish physiology–a review. *Chemosphere*, *300*, 134519.

Sharma, A., Grewal, A. S., Sharma, D., & Srivastav, A. L. 2023. Heavy metal contamination in water: consequences on human health and environment. In *Metals in water* (pp. 39–52). Elsevier.

Prabakaran, K., Sompongchaiyakul, P., Bureekul, S., Wang, X., &Charoenpong, C. 2024. Heavy metal bioaccumulation and risk assessment in fishery resources from the Gulf of Thailand. *Marine Pollution Bulletin*, *198*, 115864.

Taslima, K., Al-Emran, M., Rahman, M. S., Hasan, J., Ferdous, Z., Rohani, M. F., & Shahjahan, M. 2022. Impacts of heavy metals on early development, growth and reproduction of fish–A review. *Toxicology Reports*, *9*, 858–868.

Vasconcelos, R. T., Copatti, C. E., Albinati, A. C., Campeche, D. F. B., Bonfá, H., & Melo, J. 2024. Waterborne copper sulfate toxicity in Nile tilapia (Oreochromis niloticus) juveniles affect survival, growth, and physiology. *Journal of Applied Aquaculture*, *36*(1), 129–150.

Waichman, A. V, de Souza Nunes, G. S., de Oliveira, R., López-Heras, I., & Rico, A. 2024. Human health risks associated to trace elements and metals in commercial fish from the Brazilian Amazon. *Journal of Environmental Sciences*.

Yasmeen, R., & Rafique, S. 2024. *Assessment of Heavy Metals in Different Organs of Wallago Attu and Sperataseenghala at Different Sites of River Chenab, Pakistan*.

Zhang, J., Yang, T., Wang, N., Luo, X., Li, H., & Liao, Y. 2024. Health risk assessment of heavy metals in wild fish and seasonal variation and source identification of heavy metals in sediments: a case study of typical urban river in Xi'an, China. *Environmental Science and Pollution Research*, 1–19.

Zulfahmi, I., El Rahimi, S. A., Adani, K. H., Nasution, I. A., Nafis, B., & Utami, C. S. 2024. Reproductive performance of Zebra Fish (Danio rerio) exposed to palm oil mill eflluent in chronic toxicity. *BIO Web of Conferences*, *87*, 02004.

Annals of Plant and Soil Research 26(2):302-310(2024)
https://doi.org/10.47815/apsr.2024.10364

A comparative study of acquisition of heavy metals and its toxicological effects in fish species: *Siluriformes* and *Channa punctata*

BHARTI GUPTA[1*] AND RAMAKANT MAURYA

Maharishi University of information technology, Department of Zoology,Dubagga Lucknow 226013

Received, January, 2024; Revised accepted, May, 2024

ABSTRACT

The heavy metals damage fish's physiologic and chemical composition, causing structural damage and hinderingtheiractivities.Lucknow'sfisheriessector, accountingfor1%ofGDP,facescontaminationfromheavy metals, pesticides, and fertilizers. This study examines heavy metal levels in Siluriformes and Channa punctata fish and their buildup in the Gomati River water reservoir. Fish samples from the Gomati River near Lucknow, India, were collected and dissected into pieces with stainless steel blades. The samples were then dried at 100°C for 24 hours, revealing the anatomical features of Siluriformes and Channa punctata species. The Pb, a heavy metal, was found to have the highest concentration in the scales of Siluriformes, with values in various regions, while Cd had the lowest concentration, ranging from 0.016 to 0.112 mg/kg. Excessive consumption of fish can be dangerous due to toxic metallic elements accumulation. Siluriformes and Channa punctata fish species have high levels of Lead and Cadmium, respectively, indicating potential health risks. Channa punctata andSiluriformesfishhavehigherheavymetalconcentrations thanSiluriformes,withlead,iron,andnickellevels beyond WHO limits, while chromium, zinc, cadmium, and copper levels are below WHO limits.

Keywords: Heavy metals toxic effects,*Siluriformes,Channapunctata,*Gomati River

INTRODUCTION

Heavy metals damage fish's physiologic and chemical composition, leading to organ dysfunction and the accumulation of high-atomic weight metallic elements over time. Heavymetals in fish cause structural damage, alter condition markers, and cause genetic material damage, impacting their biodiversity, despite their high number of species. Lucknow'sfisheries sector, a subsidiary of agriculture, contributes 1% to the GDP. Utter Pradesh's freshwater resources yielded 701,726 metrictons in 2020 **(**Shahjahan *et al.,* 2022**)**. The growing population demands increased food production, making the fish industry a vitalsourceofproteinandlipid-richsustenance(Ali*et al.,* 2014).Fish lipids, rich in polyunsaturated n- 3PUFA, are essential for biological processes and nutritional value, reducing cholesterol levels and reducing heart disease risk (Zeitoun *et al.,* 2014).Followingthisstudy,fishareconsidereda viable treatment option for individuals with cardiovascular conditions (Rashed *et al.,* 2001). Thefarmingindustryiscausing water suppliesto be contaminated with hazardous contaminants like heavy metals, pesticides, and artificial fertilizers,posingsignificanthealthriskstofish consumption (Burger *et al.,* 2005; Castro-González *et al.,* 2008; Witeska *et al.,* 2005 and Gupta *et al.,* 2015).Fish bioaccumulation accumulates heavy metals in tissues, serving as a bioindicator for high atomic weight elements in water bodies, which are transferred to predators via the food chain (Tiwari *et al.,* 2014 Kumari *et al.,*2018). Pollution in fish populations reduces fertilityandaffectstheirbiologicalcharacteristics.
Elevated metals interfere with biochemistry and accumulate in aquatic organisms, damaging animal and plant tissues (Trivedi *et al.,* 2016; Singh *et al.,* 2009; Ali *et al.,* 2018 Singh *et al.,* 2016). Aquatic species experience sublethal illness in their reproductive, kidney, liver, respiration, and nervous systems as a result of heavy metal exposure (Tiwari *et al.,* 2014). Polyunsaturated fatty acids undergo membrane lipid peroxidation, causing lipid-free radicals that harm lipids, proteins, and carbohydrates. Heavy ions like Cadmium and lead in wastewater pose health risks and environmental issues (Mishra *et al.,* 2008). For intensity, Cd (II) is categorized as a class I human carcinogen, whereas Pb (II) poses a greater risk to children compared to adults since it is ingested at a higher rate (Mehmood *et al.,* 2019 and Maurya *et al.,* 2016).

**CorrespondingEmail:bg6568053@gmail.com*

MATERIALS AND METHODS

The samples of *Siluriformes* and *Channa punctata* species of fish, along with samples of water,werecollectedfromtheGomatiRivernear Lucknow, India.The water and sample sediments were separately gathered in transparent cleaned plastic bottles. The sedimentsunderwentdesiccationinanovenata temperature of 100°C for 24 hours. The fish sample was separately stored in vials filled witha 5 % strength solution of formalin. To prevent deterioration, the samples were safeguarded using cleaned polythene bags and kept at a temperature of -20°C in a deep refrigerator until they were prepared for subsequent examination. The fish samples were dissected into separate pieces, including the anatomical features of afish including the head, tails, lower abdomen, scales, fins, as well as gills, using stainless steel blades that are resistant to corrosion. Every single sample was set aside in a separate porcelain dish and thereafter transferred to the oven for drying at an oven temperature of 100°C for 24 hours.

Samplepreparation

The desiccated specimens were pulverized into tiny fragments.Every specimen underwent treatment with a volume of 10 milliliters of highly concentrated (HNO_3) and 2 millilitersof(H_2O_2),withapproximately2.0grams of each sample undergoing this treatment.The specimens underwent digestion utilizing a Janeway Model-1000 hot plate, and 6.0g ofeach sample was extracted for total lipids following the standard protocol (Siddiqui *et al.,* 2019).

Acidicdecompositionoffishandsamplesof sediment

Each fish sample, weighing 2.0g, was dried, crushed, and then deposited into a 50mL conical flask. Subsequently, 10 milliliters of concentrated HNO_3 (70%) and 2 milliliters of H_2O_2 were introduced.The flask underwent gradual heating, first at 50°C and gradually reaching 120°C within 30 minutes, utilizing a hot plate. Afterward, the process was carried out by repeatedly introducing HNO_3 and H_2O_2, witheach subsequent addition raising the temperature by 12°C.The process of digestion wasinterruptedwhenaclearsolutionappeared. Once thesamples were completely digested, the resulting solution was moved to an open container for cooling.The sample wasthereafter strained through Whatman FilterPaper 42 into sterile plastic bottles with airtight stoppers, each with a volume of 50 mL.The solution's volume was augmented to 7 mL by adding 2mLof saturatedHNO_3, andatotalof 25 mL of filtered water was introduced to the solution to achieve further dilution.All the specimens were accurately labeled and utilized for the examination of heavy metal concentrations.The entire process wasrepeated until all the specimens were disintegrated and made ready for examination using the atomic absorption spectrometer. The calibration value for every metal element was established by employing the standard solution and consistently examined to evaluate the instrument's efficacy (Zeitoun *et al.,* 2014).

Lipidextractionprocedure

After the fish organs were dried and crushed, each one was subjected to individual treatmentwith200mLofacetone.Thespecimen has a mass of 6.0 grams.Lipid extraction was performed using acetone as the solvent in a continuous extractor for a maximum period of 12 hours.The acetone was distilled utilizing arotary evaporator until only 10-15mL of acetone was left in the flask. Afterward, the leftover acetone was transferred to the beaker. Ultimately, to completely remove any remnantsof oils, the flask underwent a meticulous cleansing process using recently acquired acetone.The beaker was heated to speed up the evaporation of water and fatty acetone while ensuring aconstant temperaturethroughtheuse of a water bath.The beaker was subjected to a temperature of 80°C in the oven for 1 hour to guarantee thorough evaporation. Afterward, it was transferred to a cooler environment and measured using desiccators.According to the official analytical procedure 948 (Arisekar *et al.,* 2020 and Kumari *et al.,* 2021) of the AOAC, the total weight of the lipids is equivalent to the aggregated weight of the recovered lipids.

RESULTS

This study aimed to assess the levels of heavy metals levels in several anatomical locationsof*Siluriformes*and*Channapunctata*.

Figures 1 and 2 present the quantities of the heavy metals Pb, Cr, Cd, Ni, Zn, Cu, and Fein sixdistinct anatomical regions of thefishspecies *Siluriformes* and *Channa punctata*.Theelement Pb had the highest concentration(22.49mg kg^{-1}) out of all the heavy metals identified in the scales of *Siluriformes*. Measurements were taken to determine the metal concentrations in various regions of the *Siluriformes*. Pb exhibited the highest concentration,withvaluesof15.60,17.15, 20.20, 14.32, 20.10, and 22.50mg/kg in theskull,thegillsstomach,tail,fins,andscale, respectively. In contrast, Cd exhibited the lowest concentration, ranging from 0.016 to 0.112mg/kg in the corresponding sections. The tail of *Channa punctata* exhibited the highest concentrationoftheheavymetalPb(32.9mg/kg) among its body parts. The skull,gills stomach, tail, fins, and scale of *Channa puncta* contained several heavy metals.The concentration of Pb reached its highest levels at 21.2,30.0,22.24,32.2,25.6,and30.8mgkg^{-1}, respectively. In contrast, Cd exhibited the lowest concentrationat0.176,0.144,0.016,0.08, 0.096,and0.144mgkg^{-1},respectively.

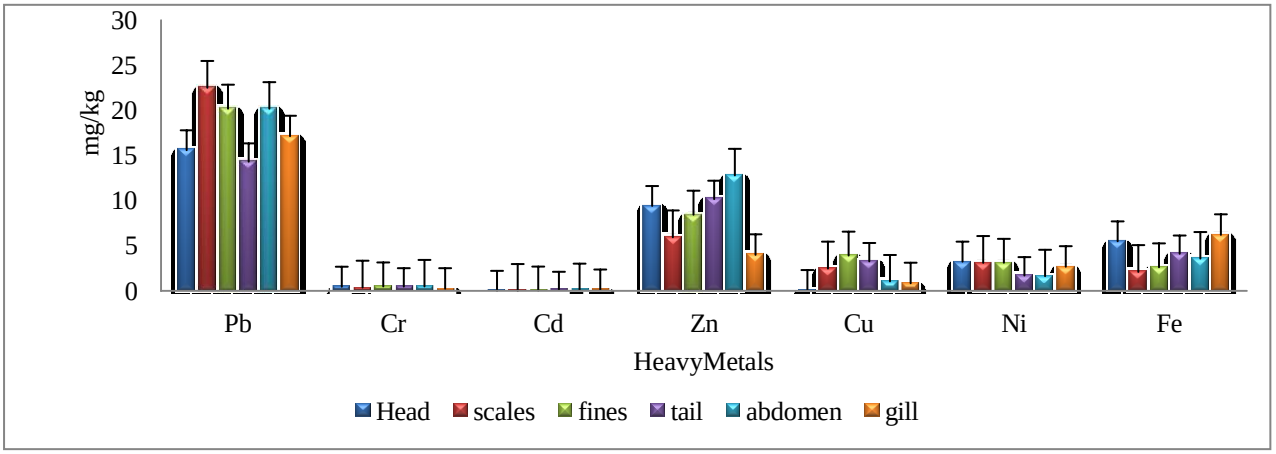

Figure1:QuantificationofheavymetallevelsinvariousorgansofSiluriformes

Figure 3 presents the level of several heavy metals detected in samples of water obtained from the Daliganj, Saheed smarak, and Hanumansetu regions along the Gomati river bank in Lucknow.The effluent water sample displayed the most elevated levels of Pb compared to other heavy metals, measuring 19.802mgL^{-1}. The recorded Pb levels in the Daliganj, Saheedsmarak, and Hanumansetu portions were 15.40±0.05, 18.10±0.08, and 19.802±0.03mg/L,correspondingly.Cadmium (Cd) had the most minimal quantity compared to other metals, with levels of 0.144±0.05, 0.16±0.01, and 0.128±0.09mg/L at the Daliganj, Saheedsmarak, and Hanumansetu sections, respectively. The sediment sample retrievedfrom the river is represented in Figure 3, which displays the levels of heavy metals detected.The element Pb had the greatest concentration, measuring 12.5mg kg^{-1}, while Cd demonstrated the lowest amount, measuring 0.16mg/kg.

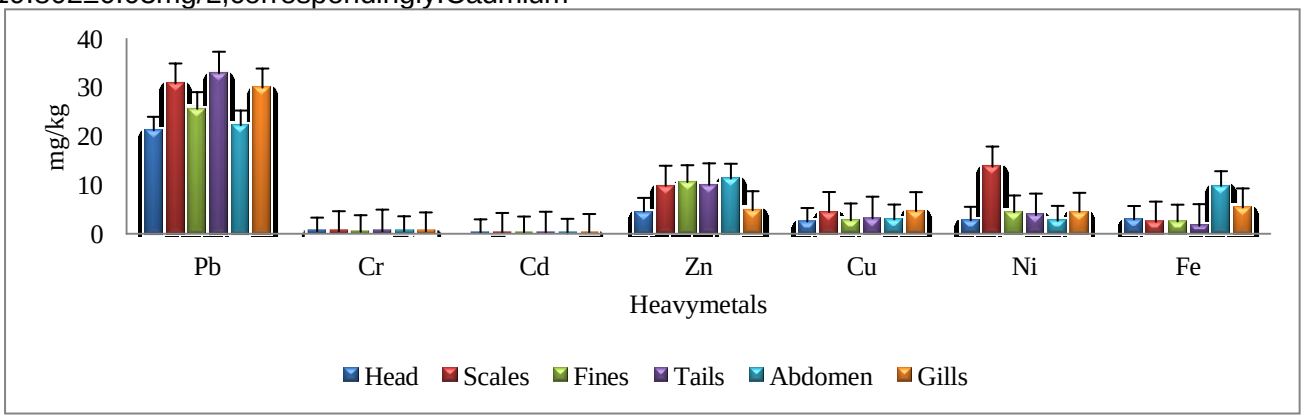

Figure2:QuantificationofheavymetallevelsinvariousorgansofChanna*punctata.*

Figure 4 displays a comparative analysis of the heavy metal concentrations for both species. The abdominal region of *Channa punctata* exhibited the highest concentration of iron across all organs in both species.The amount of iron (Fe) in the anatomical features of a fish including the skull, tails, fins, and gill of *Siluriformes* was higher compared to that of *Channa punctata*. Conversely, the iron levels in the scales and belly of *Channa punctata* were more than that in *Siluriformes*. The scales of *Channa punctata* had the most elevated content of nickel (Ni). *Channa punctata* exhibited elevated levels of nickel present in the tail, the gills the fins, along with scales when comparedto *Siluriformes*.The nickel concentration in the skull of *Siluriformes* exceeded that in *Channa punctata*.Elevated concentrations of copper (Cu) were seen in the scales, the head,stomach, and gill of *Channa punctata* in comparison to *Siluriformes*. However, the appendages located at the back of the body, specifically the fins as well as the tail, of the*Siluriformes* exhibited a much higher copper concentration in comparison to that of the *Channa punctata*. *Siluriformes* possess a higher concentration of zinc in their cranial, caudal, and abdominal regions in comparison to *Channa punctata*. However, the *Channa punctata*species displays elevated concentrations of zinc in its scales, gills, and fins in comparison to the *Siluriformes*.

Upon analyzing the cadmium content, it was observed that *Channa punctata* had higher amounts in the head, scales, gills, and fins in comparison to *Siluriformes*. In contrast, the tail and abdomen of *Siluriformes* exhibited elevated cadmium levels compared to *Channa punctata*. Chromium concentrationsinthehead, abdomen, and gills of *Channa punctata* were found to be elevated in comparison to *Siluriformes*. In contrast, the concentrations of chromium in the fins and tail of *Channa punctata* were comparatively lower than those found in *Siluriformes.* Nevertheless, both species displayed comparable quantities of chromium in their scales.The concentration of lead was higher in all six organs of Siluriformes when comparedto *Channapunctata. Channapunctata* had a higher quantity of heavy metals, specifically lead, in comparison to *Siluriformes*. Figure 5 displays a comparative analysis of the lipid proportions in both species. The*Siluriformes* species had the largest lipid proportion, measuring 39.34 percent, while the *Channa punctata* species had the smallest lipid proportion, detecting 8.56 percent.The documented lipid content in all six body segments of both species is as follows:scales< fins<tail<abdomen<gills<head. The comprehensive research indicated that the total amount the lipid content in each of the sixtissues was higher in *Siluriformes* compared to Channa *punctata*.

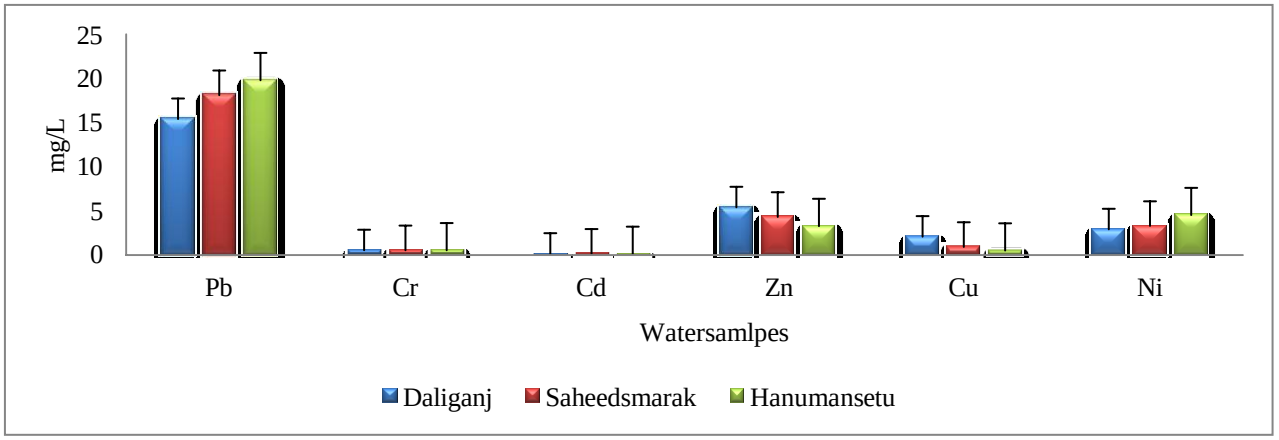

Figure3:WatersampleswereretakenfromthreeseparatelocationsalongtheGomatiRivertomeasure the levels of heavy metals

DISCUSSION

Thisinvestigationfocusedontwotypes offish,specifically*Siluriformes*and*Channa punctata*, which were found in the Gomati River in Lucknow.Scientific research suggests that consuming an excessive number of fish can be dangerousbecauseoftheaccumulationoftoxic

metallic elements.Therefore, it was imperative to evaluate the existence of toxic metals in the fish species *Siluriformes* and *Channa punctata.* The concentrations of metallic elements and the amount of lipids in both of these species were examined and displayed in Figure 1-5.Thefigure presents the concentrations of several heavy metals detected in different anatomical regionsof *Siluriformes*and*Channapunctata*fish species collected from the Gomati River in Lucknow.The element Lead (Pb) exhibits the most elevated level among *Siluriformes* scales, specifically measuring 22.5mg/kg. On the other hand, cadmium (Cd) has the smallest amount, measuring 0.016mg/kg, among all the heavy metals that are present.The *Siluriformes* had the highest levels of Pb concentration in several body parts, specifically the head (15.60mgkg^{-1}), gills (17.15mgkg^{-1}),belly (20.20mgkg^{-1}),tails (14.32mgkg^{-1}), fins (20.10mgkg^{-1}), and scales (22.50mgkg^{-1}). Conversely, Cd exhibited the lowestlevel(0.032,0.112,0.112,0.096,0.064, and 0.016mgkg^{-1}) in the corresponding anatomical regions.The levels of lead, nickel, and iron, as specified by the WHO, were above the permissible limits, whereas the quantities of Cr, Cu, Zn, and Cdwere within the acceptable range (Kumari *et al.,*2019; Kumar *et al.,* 2020; Siraj *et al.,* 2014andAhmed*et al.,* 2019). Figure 1 presents the amounts of heavy metal contentin different anatomical regions of the *Channa punctat.* Among all the elements examined, the tail of *Channa punctata* contained the highest concentration of lead (Pb) at 32.9mgkg^{-1}, while the stomach had the lowest level of cadmium (Cd) at 0.016 mgkg^{-1}.

An examination of heavy metal concentrations in several body sections of *Channa punctata* demonstrated that the scales, fins,tails,abdomen,andheadexhibitedelevated levels of lead, with respective values of 30.8, 25.6,32.2,22.24,30.0,and21.2mgkg^{-1}.In contrast, these components exhibited the most minimallevelsofCd,at0.176,0.144,0.016, 0.08, 0.096, and 0.144mg/kg, respectively. The concentrations of lead in different anatomical areasof*Channapunctata*rangedfrom21.2to 32.9 mg/kg. The levels of lead (Pb), iron (Fe), and nickel (Ni) were above the permissible thresholds, but Cd, Zn, and Cr stayed within the permissible limits. Conversely, Cu remained within the allowable limits threshold as per the WHO criterion. The current investigationunveiled distinct concentrations of metals in the interior tissues of both animals. Kalay *et al.,* 1999, noted that various piscine species display diverse degrees of metal concentration within their bodily tissues.Moreover, Canli *et al.,*2003, have recorded that the levels of heavy metal content in fish display fluctuations depending on the species and the specific aquatic habitat they reside.Kamaruzzaman *et al.,* 2010, noted a significant increase in the concentrations of Pb and Cd in tissues of *Cyprinus carpio* for all heavy metals. Figure 2 presents the levels of heavy metals in threewater samples (Daliganj, Saheedsmarak, and Hanumansetu) obtained from the Gomati River Lucknow. The exit water sample exhibits the most elevated level of lead (Pb) content compared to all other metals, particularly measuring 19.802 mg/kg.

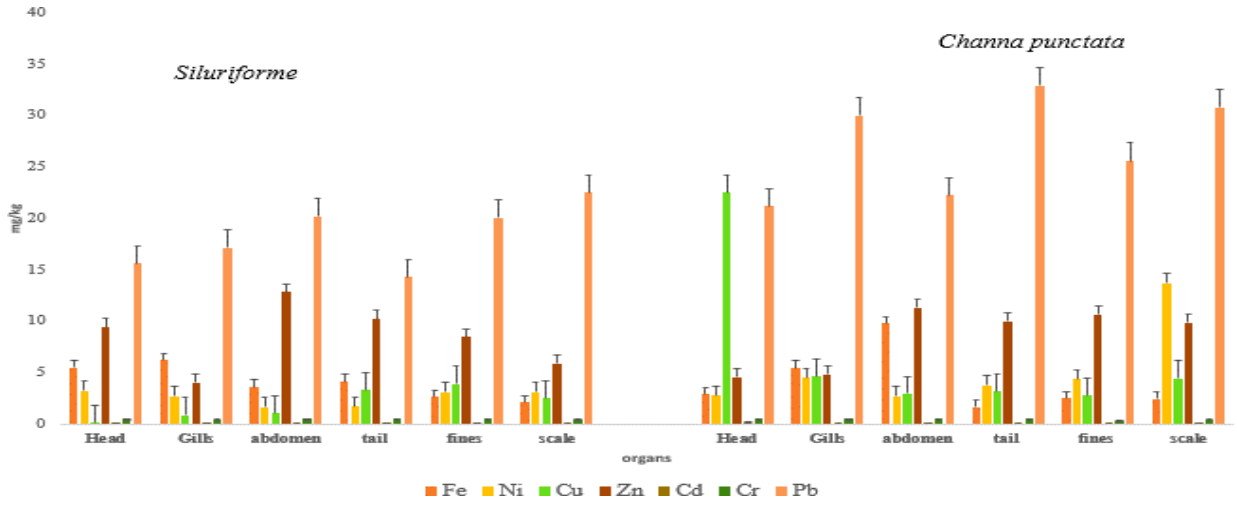

Figure4:Acomparativeanalysisoftheheavymetalconcentrationsforbothspecies

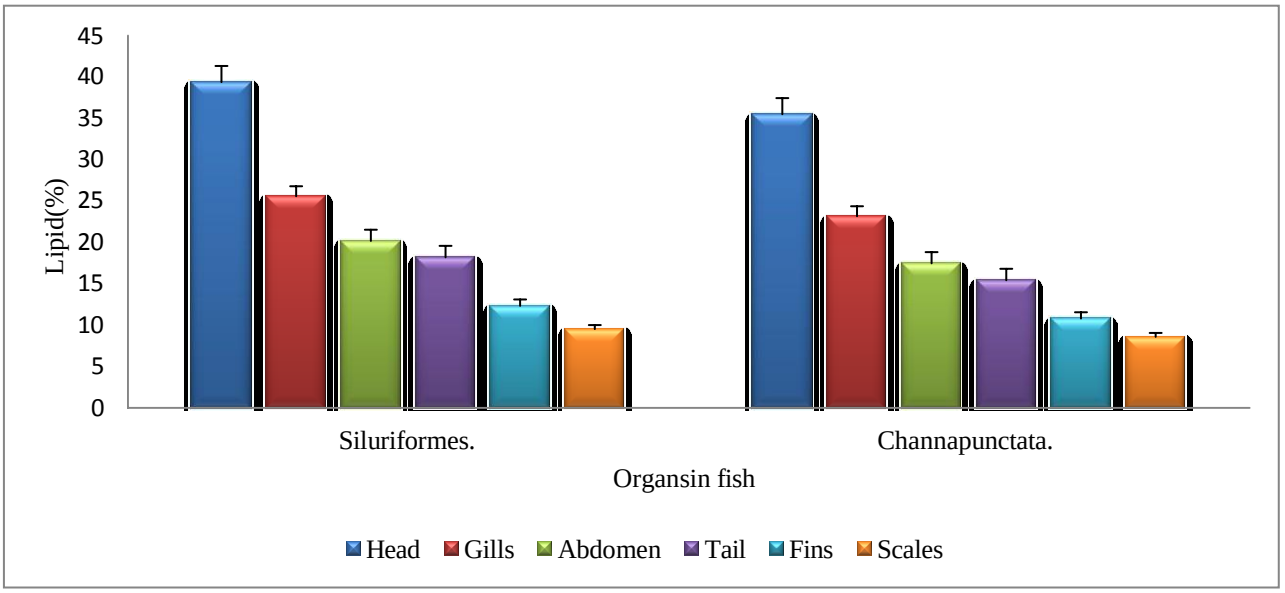

Figure5:Comparativeanalysisoflipid%indifferentbodypartsoffish

The recorded levels of metallic elements with high atomic weights in the primary Hanumansetu, Saheedsmarak, and Daliganj,and regions were as stated:The element Pbhad the highest quantity, measuring 19.802±0.10,18.10±0.14,and15.40±0.18mg/L, correspondingly. In contrast, Cd exhibited the most minimal quantity compared to the other metals, with measurements of 0.128±0.05, 0.16±0.17, and0.144±0.14mg/L, respectively. The metals in the Hanumansetu, water region were found to have the following concentration sequence: Cd < Cr < Cu < Ni < Fe< Zn < Pb.The concentration order in the Saheedsmarak water zone was as follows: Cd < Cr < Cu< Fe < Ni < Zn < Pb.The Daliganj, water zone was found to have heavy metal concentrations in the following order: Cd< Cu < Cr< Fe < Zn < Ni <Pb.All metals were present in the water above the standard threshold established by the World Health Organization, except for Cu.

Figure 3 displays the levels of levels of Fe, Ni, Cd, Zn, Cu Cr, and Pbfound in the sediment sample taken from Gomati River Lucknow. Lead has the largest concentration among these elements, measuring 12.5 mg/kg, whilst Cd has the lowest level, measuring 0.16 mg/kg.Analyzed sediment samples revealed metal levels, ranked in decreasing order of intensity (mg/kg), which were as follows: Pb, Ni, Zn, Cu, Fe, Cr, and Cd.The levels of iron, cadmium, lead, and copper amounts surpassed the permissible barrier, whereas the Zn, Cr, and Nilevelspersistedwithinthelimitestablishedby the World Health Organization. Establishing an association between the levels of heavy metalsis a significant challenge, particularly when comparing the same organs among different species.These variations are determined by factors such as dietary habits, the fish's ecosystem being located in extremely deep locations feeding behavior, and age. Kamaruzzaman *et al.,* 2010, discovered the relationship between the concentration of metals and other important characteristics of fish, such as their size, age, and genetic composition, which is being examined for association. Figure4 displays a level comparison of (Fe), (Ni), (Cu), (Zn), (Cd), (Cr), and (Pb)in the two species. The abdominal region of *Channa punctata* displayed the highest iron concentration compared to all other organs, at precisely 9.82mg/kg.The *Siluriformes* exhibited a higher concentrationthan Channa punctata in the head, neck, tail,and wings.The *Channa punctata* species exhibited higher levels of abdomen and scale Fe content compared to the *Siluriformes* species. The scales of *Channa punctata* showed the highest concentration among all the organs, with a precise measurement of 13.74 mg/kg. The *Channa punctata* had higher nickel levels in its fins, tail, scales, and gills in comparison to the *Siluriformes*.Thenickelconcentrationintheskull of *Siluriformes* exceeded that in *Channa punctata*. *Channa punctata* had elevated copper levels in the head, abdomen, gills, and scales in comparison to *Siluriformes*. In contrast, *Siluriformes*exhibitedgreatercopper

concentrations in their fins and tails compared to *Channa punctata*.Thezinc concentration in the head, abdomen, and tail of *Siluriformes* was greater in comparison to *Channa punctata*. *Channa punctata* had a greater zinc concentration in the gills, scales, and fins compared to *Siluriformes.*

The *Channa punctata* had higher concentrations of cadmium (Cd) in the skull, scales, fins, and gills in comparison to *Siluriformes*. In contrast, the tail and abdomen of *Siluriformes e*xhibited greater levels of cadmium comparedto*Channapunctata.Channapunctata* exhibited higher concentrations of Cr in thehead, abdomen, and gills compared to *Siluriformes*. Nevertheless, the fins and tail of *Siluriformes* exhibited a greater chromium concentration in comparison to *Channapunctata*.Both species possess scales with the same concentration of chromium. *The Channa punctata*showedhigherlevelsofleadinall 6organs compared to the *Siluriformes*. *Channa punctata* had a higher overall concentration of heavy metals, specifically lead, in comparison to *Siluriformes*.The occurrence can be ascribed to the inflow of water from the catchment area of District Lucknow, lead he inundation of the Gomati Riverwith excessive amounts of water. TheheightenedlevelofPbisascribedtothe erosioncausedbyunidentifiedmountainsand valleys.

CONCLUSIONS

Studies have shown that the accumulation of heavy metals causes adecrease in the lipid composition of fish.The heavy metal concentration in *Channa punctata* exceeded that in *Siluriformes,* despite *Channa punctata* possessing a lower total lipid % in comparison to *Siluriformes.* Both fish species exhibited lead, iron, and nickel concentrations that were beyond the permissible range set by WHO. In contrast, chromium (Cr), zinc (Zn), cadmium (Cd), and copper (Cu) were found tobe below the allowable limit.The metal concentrationsexhibitedvariationamongseveral anatomical locations of both fish species, as evidenced in figure 1 and 2.This fact demonstrates the magnitude of heavy metal accumulation inthetissues of manyfishspecies.

ACKNOWLEDGMENT

The author would like to thank, the Department of Zoology, Maharishi University of Information Technology, Dubagga, Lucknow (Uttar Pradesh) India. For their guidance and support in completing this article.

REFERENCES

Ahmed,A.S.,Sultana,S.,Habib,A.,Ullah,H., Musa,N.,Hossain,M.B.,&Sarker,M.S. I. (2019) Bioaccumulation of heavy metals in some commercially important fishes from a tropical river estuary suggests higherpotentialhealthriskinchildrenthan adults. *Plos one*, *14*(10), e0219336.

Ali, A. S., & US SA, A. R. (2014) Effect of different heavy metal pollution on fish. *Res. J. Chem. Environ. Sci*, **2**(1), 74-79.

Ali, D., Almarzoug, M. H., Al Ali, H., Samdani, M. S., Hussain, S. A., & Alarifi, S. (2020) Fish as bio indicators to determine the effectsof pollution in river by using the micronucleus and alkaline single cell gel electrophoresis assay. *Journal of King SaudUniversity-Science*,**32**(6),2880-2885.

Ali,H.,&Khan,E.(2018)Assessmentofpotentiallytoxicheavymetalsandhealth risk in water, sediments, and different fish species of River Kabul, Pakistan. *Human and Ecological Risk Assessment: An International Journal*, **24**(8), 2101-2118.

Arisekar, U., Shakila, R. J., Shalini, R., & Jeyasekaran,G.(2020)Humanhealthrisk assessment of heavy metals in aquatic sedimentsandfreshwaterfishcaughtfrom Thamirabarani River, the Western Ghatsof South Tamil Nadu. *Marine Pollution Bulletin*, **159**, 11149

Burger, J., & Gochfeld, M. (2005) Heavy metals in commercial fish in New Jersey. *Environmental research*, *99*(3), 403-412.Castro-González, M. I., & Méndez-Armenta, M. (2008). Heavy metals: Implications associated to fish consumption. *Environmental toxicology and pharmacology*, **26**(3), 263-271.

Canli, M., & Atli, G. (2003) The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. *Environmental pollution*, **121**(1), 129-136.

Domingo, J. L. (2007) Omega-3 fatty acids and the benefits of fish consumption: is all that glitters gold?. *Environment international*, **33**(7), 993-998.

Gaur, V. K., Gupta, S. K., Pandey, S. D., Gopal, K., & Misra, V. (2005). Distribution of heavy metals in sediment and water of riverGomti.*Environmentalmonitoringand assessment*, **102**, 419-433.

Gupta,S.K.,Chabukdhara, M., Singh,J., &Bux, F. (2015) Evaluation and potential health hazard of selected metals in water, sediments, and fish from the Gomti River. *Human and Ecological Risk Assessment: An International Journal*, **21**(1), 227-240.

Juturu, V. (2008) Omega-3 fatty acids and the cardiometabolic syndrome. *Journal of the cardiometabolic syndrome*, **3**(4), 244-253.

Kalay, M., Ay, Ö., & Canli, M. (1999) Heavy metal concentrations in fish tissues from the Northeast Mediterranean Sea. *Bulletin of environmental contamination and toxicology*, **63**, 673-681.

Kamaruzzaman, B. Y., Ong, M. C., & Rina, S. Z. (2010) Concentration of Zn, Cu and Pb in some selected marine fishes of the Pahang coastal waters, Malaysia. *American journal of applied sciences*,**7**(3), 309-314.

Kumar, A., Kumar, A., & Jha, S. K. (2020) Seasonal pollution of heavy metals in water, sediment, and tissues of catfish (Heteropneustes fossilis) from Gogabil Lake of north Bihar, India. *India International Journal of Fisheries and Aquatic Studies*, **8**(2), 163-175.

Kumari, P., & Maiti, S. K. (2019)Health risk assessment of lead, mercury, and other metal (loid) s: A potential threat to the population consuming fish inhabiting, a lentic ecosystem in Steel City (Jamshedpur), India. *Human and Ecological Risk Assessment: An International Journal*.

Kumari, P., & Maiti, S. K. (2021) Bioaccessibilities and health risk assessment of heavy and trace elements infishfromanurbancity,India.*Human and Ecological Risk Assessment: An International Journal*, **27**(1), 50-70.

Kumari, P., Chowdhury, A., & Maiti, S. K. (2018) Assessment of heavy metal in the water, sediment, and two edible fish species of Jamshedpur Urban Agglomeration, India with special emphasis on human health risk. *Human and Ecological Risk Assessment: An International Journal*, **24**(6), 1477-1500.

Levesque,H.M.,Moon,T.W.,Campbell,P.G.C., & Hontela, A. (2002) Seasonal variation in carbohydrate and lipid metabolism of yellow perch (Perca flavescens) chronically exposed to metals in the field. *Aquatic Toxicology*, **60**(3-4), 257-267.

Maurya, P. K., & Malik, D. S. (2016) Distribution of heavy metals in water, sediments and fish tissue (Heteropneustis fossilis) in Kali River of western UP India. *International Journal of Fisheries and Aquatic Studies*, **4**(2), 208-215.

Mehmood,M.A.,Qadri,H.,Bhat,R.A.,Rashid, A., Ganie, S.A., & Dar, G.H. (2019) Heavy metal contamination in two commercialfish species of a trans-Himalayan freshwater ecosystem. *Environmental monitoring and assessment*, **191**(2), 104.

Mishra,S.S.,&Mishra,A.(2008)Assessmentof physico-chemical properties and heavy metal concentration in Gomati river. *Research in Environment and Life Sciences*, **1**(2), 55-58.

Shahjahan, M., Taslima, K., Rahman, M. S., Al-Emran, M. D., Alam, S. I., & Faggio, C. (2022) Effects of heavy metals on fish physiology–a review. *Chemosphere*, **300**, 134519.

Siddiqui,E.,Verma,K.,Pandey,U.,&Pandey, J. (2019) Metal contamination in seven tributaries of the Ganga River and assessment of human health risk fromfish consumption. *Archives of environmental contaminationandtoxicology*,**77**,263-278.

Sidhu, K. S. (2003) Health benefits and potential risks related to consumption of fish or fish oil. *Regulatory toxicology and pharmacology*, **38**(3), 336-344.

Singh, B. P., & Tandon, P. K. (2009) Effect of river water pollution on hematological parameters of fish, Wallagoattu. *Res Environ Life Sci*, **2**(4), 211-214.

Singh, V. P., Raghuvanshi, A. S., Singh, P., Singh, S.K., & Singh, A.K. (2016) Assessment of water quality in the river gomati at Jaunpur (UP), India. *Annals of Plant Sciences*, **5**(3), 1312-1317.

Siraj, M., Shaheen, M., Sthanadar, A. A., Khan, A., Chivers, D. P., & Yousafzai, A. M. (2014) A comparative study of bioaccumulation of heavy metals in two freshwaterspecies,Aorichthysseenghala and Ompok bimaculatous at River Kabul, KhyberPakhtunkhwa,Pakistan.*Journalof Biodiversity and Environmental Sciences*, **4**(3), 40-54.

Sobha,K.,Poornima,A.,Harini,P.,&Veeraiah, K. (2007) Astudyonbiochemicalchanges in the fresh water fish, Catla catla (Hamilton) exposed to the heavy metal toxicant cadmium chloride. *Kathmandu university journal of science, engineering and technology*, **3**(2), 1-11.

Tiwari, A., & Dwivedi, A. C. (2014) Assessment of heavy metals bioaccumulation in alien fish species Cyprinus carpio from the Gomti river, India. *European Journal of Experimental Biology*, **4**(6), 112-117.

Tiwari, A., Dwivedi, A. C., Shukla, D. N., & Mayank, P. (2014) Assessment of heavy metals in different organ of Oreochromis niloticus from the Gomti river at Sultanpur, India. *Journal of the Kalash Science*, **2**(1), 47-52.

Trivedi, P., Singh, A., Srivastava, A., Sharma, V. P., Pandey, C. P., Srivastava, L. P., & Malik, S. (2016) An assessment of water quality of Gomati River particular relevant to physicochemical characteristics, pesticide and heavy metal. *Int J Eng Res Appl*, **6**(9), 66-75.

Witeska, M. (2005) Stress in fish-hematological and immunological effects of heavy metals. *Electronic journal of ichthyology*, **1**(1), 35-41.

Zeitoun, M. M., & Mehana, E. E. (2014) Impactof water pollution with heavy metals onfish health: overview and updates. *Global veterinaria*, *12*(2), 219-231.Rashed, M. N. (2001) Monitoring of environmental heavy metals in fish from Nasser Lake. *Environment international*, **27**(1), 27-33.

Thesis_Bharti

ORIGINALITY REPORT

15%	11%	10%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	dergipark.gov.tr Internet Source	1%
2	Md Shahjahan, Khanam Taslima, Mohammad Shadiqur Rahman, Md Al-Emran, Shanon Iffat Alam, Caterina Faggio. "Effects of heavy metals on fish physiology – A review", Chemosphere, 2022 Publication	1%
3	krishikosh.egranth.ac.in Internet Source	1%
4	www.researchgate.net Internet Source	1%
5	jabonline.in Internet Source	1%
6	link.springer.com Internet Source	1%
7	patents.glgoo.top Internet Source	<1%
8	myscholar.umk.edu.my Internet Source	

		<1%
9	Submitted to Higher Education Commission Pakistan Student Paper	<1%
10	Ju-Wook Lee, Hoon Choi, Un-Ki Hwang, Ju-Chan Kang, Yue Jai Kang, Kwang Il Kim, Jun-Hwan Kim. "Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review", Environmental Toxicology and Pharmacology, 2019 Publication	<1%
11	Ramsha Khan, Abhishek Saxena, Saurabh Shukla. "Evaluation of heavy metal pollution for River Gomti, in parts of Ganga Alluvial Plain, India", SN Applied Sciences, 2020 Publication	<1%
12	www.patentguru.com Internet Source	<1%
13	www.researchsquare.com Internet Source	<1%
14	dyuthi.cusat.ac.in Internet Source	<1%
15	www.coursehero.com Internet Source	<1%

16	Suryapratap Ray, Rahul Vashishth. "From Water to Plate: Reviewing the Bioaccumulation of Heavy Metals in Fish and Unraveling Human Health Risks in the Food Chain", Emerging Contaminants, 2024 Publication	<1 %
17	Submitted to University of Minnesota System Student Paper	<1 %
18	etheses.whiterose.ac.uk Internet Source	<1 %
19	ujdigispace.uj.ac.za Internet Source	<1 %
20	eprints.hud.ac.uk Internet Source	<1 %
21	Heri B. Santoso, Eko Suhartono, Rizmi Yunita, Danang Biyatmoko. "Mudskipper Fish as a Bio-indicator for Heavy Metals Pollution in a Coastal Wetland", Egyptian Journal of Aquatic Biology and Fisheries, 2020 Publication	<1 %
22	en.wikipedia.org Internet Source	<1 %
23	worldwidescience.org Internet Source	<1 %
24	backend.orbit.dtu.dk Internet Source	<1 %

25	K. Balamurugan. "Stimulatory effect of the ethanol extract of Melastoma malabathricum L. (Melastomataceae) leaf on the reproductive system of male albino rats", Journal of Applied Pharmaceutical Science, 2013 Publication	<1 %
26	Submitted to University of Huddersfield Student Paper	<1 %
27	www.sciencepub.net Internet Source	<1 %
28	www.frontiersin.org Internet Source	<1 %
29	Farhan Jamil Emon, Md Fazle Rohani, Nusrat Sumaiya, Mst Fatema Tuj Jannat et al. "Bioaccumulation and Bioremediation of Heavy Metals in Fishes—A Review", Toxics, 2023 Publication	<1 %
30	oar.icrisat.org Internet Source	<1 %
31	"Phytoremediation", Springer Science and Business Media LLC, 2016 Publication	<1 %
32	Submitted to Monmouth University Student Paper	<1 %
	pdf.usaid.gov	

33	Internet Source	<1%
34	www.ncbi.nlm.nih.gov Internet Source	<1%
35	www.science.gov Internet Source	<1%
36	Smiti Kumar, Garima Chawla, Arvind K. Bansal. "Spherical Crystallization of Mebendazole to Improve Processability", Pharmaceutical Development and Technology, 2008 Publication	<1%
37	Moumita De, Mazlan Abd. Ghaffar, Noorashikin Md. Noor, Zaidi Che Cob, Yosni Bakar, Simon Kumar Das. "Effects of water temperature and diet on blood parameters and stress levels in hybrid grouper (Epinephelus fuscoguttatus ♀ × E. lanceolatus ♂) juveniles", Aquaculture Reports, 2019 Publication	<1%
38	mts.intechopen.com Internet Source	<1%
39	Ana Picado, Susana M. Paixão, Liliana Moita, Luis Silva et al. "A multi-integrated approach on toxicity effects of engineered TiO2 nanoparticles", Frontiers of Environmental Science & Engineering, 2015	<1%

	Publication	
40	ir.library.ui.edu.ng Internet Source	<1 %
41	phaidra.univie.ac.at Internet Source	<1 %
42	scholar.smu.edu Internet Source	<1 %
43	tu-freiberg.de Internet Source	<1 %
44	digitalarchive.boun.edu.tr Internet Source	<1 %
45	Submitted to Delhi Technological University Student Paper	<1 %
46	Atoosa Haghighizadeh, Omid Rajabi, Arman Nezarat, Zahra Hajyani et al. "Comprehensive analysis of heavy metal soil contamination in mining Environments: Impacts, monitoring Techniques, and remediation strategies", Arabian Journal of Chemistry, 2024 Publication	<1 %
47	B Mahalakshmi, V Sakthivel, B Sumitha Devi, S Swetha. "Agricultural Crop and Fertilizer Recommendations based on Various Parameters", 2023 International Conference on Sustainable Computing and Smart Systems (ICSCSS), 2023	<1 %

Publication

48	Submitted to University of Gloucestershire Student Paper	<1 %
49	Xiangyang Wu, Samuel J. Cobbina, Guanghua Mao, Hai Xu, Zhen Zhang, Liuqing Yang. "A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment", Environmental Science and Pollution Research, 2016 Publication	<1 %
50	Dharmendra Kumar, Dinabandhu Sahoo. "Assessment of Physiochemical Parameters and Bioremediation of Complex Contaminated Yamuna River, India: An Algal-Based Approach", Water, Air, & Soil Pollution, 2024 Publication	<1 %
51	Submitted to Universiti Teknologi Malaysia Student Paper	<1 %
52	baadalsg.inflibnet.ac.in Internet Source	<1 %
53	www2.mdpi.com Internet Source	<1 %
54	Megha Mittal, S. J. S. Flora. "Vitamin E Supplementation Protects Oxidative Stress During Arsenic and Fluoride Antagonism in	<1 %

Male Mice", Drug and Chemical Toxicology, 2008
Publication

55	moam.info Internet Source	<1%
56	Ming Hung Wong, Tatiana Minkina, Nikita Vasilchenko, Svetlana Sushkova et al. "Assessment of Antibiotic Resistance Genes in Soils Polluted by Chemical and Technogenic Ways with Poly-Aromatic Hydrocarbons and Heavy Metals", Environmental Research, 2024 Publication	<1%
57	Sadia Sharmin, Md Touhidul Islam, Md Anwar Sadat, Rayeda Jannat, Md Rushna Alam, Md Shahjahan. "Sumithion induced structural erythrocyte alteration and damage to the liver and kidney of Nile tilapia", Environmental Science and Pollution Research, 2021 Publication	<1%
58	what-when-how.com Internet Source	<1%
59	wildlife-biodiversity.com Internet Source	<1%
60	"Anthropogenic Pollution of Aquatic Ecosystems", Springer Science and Business Media LLC, 2021 Publication	<1%

61	Benjamin O. Botwe. "Heavy metal concentrations in five fish species from the Gulf of Guinea and their human health implications", Regional Studies in Marine Science, 2021 Publication	<1 %
62	Submitted to Korea National University of Transportation Student Paper	<1 %
63	Pallab Shaw, Paritosh Mondal, Arindam Bandyopadhyay, Ansuman Chattopadhyay. "Environmentally relevant concentration of chromium induces nuclear deformities in erythrocytes and alters the expression of stress-responsive and apoptotic genes in brain of adult zebrafish", Science of The Total Environment, 2020 Publication	<1 %
64	researchspace.ukzn.ac.za Internet Source	<1 %
65	Laís Fernanda Oya-Silva, Taynah Vicari, Geonildo Rodrigo Disner, Juliana Roratto Lirola et al. "Tissue-specific genotoxicity and antioxidant imbalance of titanium dioxide nanoparticles (NPTiO2) and inorganic lead (PbII) in a neotropical fish species", Environmental Toxicology and Pharmacology, 2021	<1 %

Publication

66	Osama E. A. Attia. "Sedimentological characteristics and geochemical evolution of Nabq sabkha, Gulf of Aqaba, Sinai, Egypt", Arabian Journal of Geosciences, 2011 Publication	<1 %
67	onlinelibrary.wiley.com Internet Source	<1 %
68	pdffox.com Internet Source	<1 %
69	Mehjbeen Javed, Nazura Usmani. "An Overview of the Adverse Effects of Heavy Metal Contamination on Fish Health", Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 2017 Publication	<1 %
70	Muhib Zaman, Fahim Ullah Khan, Waqar Younas, Muhammad Noorullah, Imdad Ullah, Li'ang Li, Amina Zuberi, Youji Wang. "Physiological and histopathological effects of polystyrene nanoparticles on the filter-feeding fish Hypophthalmichthys molitrix", Science of The Total Environment, 2024 Publication	<1 %
71	ir.uew.edu.gh:8080 Internet Source	<1 %

72	libweb.kpfu.ru Internet Source	<1 %
73	ujcontent.uj.ac.za Internet Source	<1 %
74	www.researchjournal.co.in Internet Source	<1 %
75	Caroline da Silva Montes, Maria Auxiliadora Pantoja Ferreira, Tommaso Giarrizzo, Lílian Lund Amado, Rossineide Martins Rocha. "Evaluation of metal contamination effects in piranhas through biomonitoring and multi biomarkers approach", Heliyon, 2020 Publication	<1 %
76	Hai-ping Yuan. "Antimicrobial activity of isolate HL-12 against Clavibacter michiganensis subsp. michiganensis in the presence of cadmium", Ecotoxicology, 05/2009 Publication	<1 %
77	M. Sharma, R. Kant, A. K. Sharma, A. K. Sharma. "Exploring the impact of heavy metals toxicity in the aquatic ecosystem", International Journal of Energy and Water Resources, 2024 Publication	<1 %
78	umpir.ump.edu.my Internet Source	<1 %

79	"Lead Toxicity: Challenges and Solution", Springer Science and Business Media LLC, 2023 Publication	<1 %
80	Submitted to University of Wales, Bangor Student Paper	<1 %
81	businessdocbox.com Internet Source	<1 %
82	uwspace.uwaterloo.ca Internet Source	<1 %
83	Arkajyoti Mukherjee, Gourav Dhar Bhowmick. "Sources of Heavy Metal Contamination in Surface Waters of Indian Freshwater Ecosystems and Their Effect on Aquatic Biota: A State of the Art Review", Journal of Hazardous, Toxic, and Radioactive Waste, 2024 Publication	<1 %
84	Chizoba Henrietta UNAEZE, Yvonne Adaobi ONMONYA,, David EBI, Hajara Oyiza YUSUF. "Heavy Metal Contamination in Selected Fishes from Ojo, Lagos Nigeria", Environmental Studies Journal, 2023 Publication	<1 %
85	Submitted to University of Bradford Student Paper	<1 %

86	www.tandfonline.com Internet Source	<1%
87	"Riverine Ecosystem Management", Springer Science and Business Media LLC, 2018 Publication	<1%
88	Submitted to Kwame Nkrumah University of Science and Technology Student Paper	<1%
89	Saranya Elumalai, Kolandhasamy Prabhu, Gopi Palani Selvan, Pasiyappazham Ramasamy. "Review on heavy metal contaminants in freshwater fish in South India: current situation and future perspective", Environmental Science and Pollution Research, 2023 Publication	<1%
90	Submitted to University of Malawi - The Polytechnic Student Paper	<1%
91	Submitted to University of the Philippines Los Banos Student Paper	<1%
92	Yukti Sharma, Somia Bashir, M. Irshad, S. Datta Gupta, T.D. Dogra. "Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats", Toxicology, 2005	<1%

	Publication	
93	Submitted to ebsu Student Paper	<1%
94	journal.ipb.ac.id Internet Source	<1%
95	journals.sagepub.com Internet Source	<1%
96	publications.lnu.edu.ua Internet Source	<1%
97	repository.sustech.edu Internet Source	<1%
98	repository.suza.ac.tz:8080 Internet Source	<1%
99	"Fresh Water Pollution Dynamics and Remediation", Springer Science and Business Media LLC, 2020 Publication	<1%
100	Chidambaram Jayaseelan, Abdul Abdul Rahuman, Rajendiran Ramkumar, Pachiappan Perumal et al. "Effect of sub-acute exposure to nickel nanoparticles on oxidative stress and histopathological changes in Mozambique tilapia, Oreochromis mossambicus", Ecotoxicology and Environmental Safety, 2014 Publication	<1%

101	Naveed Nabi, Imtiaz Ahmed, Gohar Bilal Wani. "Hematological and serum biochemical reference intervals of rainbow trout, Oncorhynchus mykiss cultured in Himalayan aquaculture: Morphology, morphometrics and quantification of peripheral blood cells", Saudi Journal of Biological Sciences, 2022 Publication	<1 %
102	civilejournal.org Internet Source	<1 %
103	hal-ineris.ccsd.cnrs.fr Internet Source	<1 %
104	hydromaxusa.com Internet Source	<1 %
105	ikee.lib.auth.gr Internet Source	<1 %
106	mro.massey.ac.nz Internet Source	<1 %
107	shodhgangotri.inflibnet.ac.in Internet Source	<1 %
108	www.rrcap.ait.asia Internet Source	<1 %
109	123docz.net Internet Source	<1 %
110	Submitted to Napier University	

Student Paper

<1%

---

111 R. Vinodhini, M. Narayanan. "Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp)", International Journal of Environmental Science & Technology, 2008

Publication

<1%

---

112 Runbo Li, Yinghu Hao, Yubang Shen, Lang Gui, Wenyao Lv, Li Yuan, Biao Du, Lingli Xie, Jiale Li, Xiaoyan Xu. "Impact of Cadmium and Diclofenac Exposure on Biochemical Responses, Transcriptome, Gut Microflora, and Growth Performance in Grass Carp (Ctenopharyngodon idella)", Chemosphere, 2024

Publication

<1%

---

113 Sandeep K. Mishra, Mahendra Kumar Hidau, Shivika Rai. "Memantine treatment exerts an antidepressant-like effect by preventing hippocampal mitochondrial dysfunction and memory impairment via upregulation of CREB/BDNF signaling in the rat model of chronic unpredictable stress-induced depression", Neurochemistry International, 2021

Publication

<1%

---

assets.researchsquare.com

114	Internet Source	<1%
115	dalspace.library.dal.ca Internet Source	<1%
116	jazindia.com Internet Source	<1%
117	jifro.ir Internet Source	<1%
118	pubs.sciepub.com Internet Source	<1%
119	repositorio.ufsm.br Internet Source	<1%
120	vdoc.pub Internet Source	<1%
121	www.aquast.org Internet Source	<1%
122	www.asjp.cerist.dz Internet Source	<1%
123	www.dovepress.com Internet Source	<1%
124	www.yumpu.com Internet Source	<1%

Exclude quotes	On	Exclude matches	< 10 words
Exclude bibliography	On		