Received: 10 February 2024 | Revised: 23 May 2024 | Accepted: 29 May 2024

https://doi.org/10.56946/jzs.v2i1.325

Journal of Zoology and Systematics

Review

Heavy Metals Cause Toxicity, Histopathological Abnormalities and Oxidative Stress in Major Carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*)

Maria Saeed Khan[1,*], Abdul Ghaffar[1,*], Habiba Jamil[1], Shumaila Khalid[2], Batha Tafazul[1]

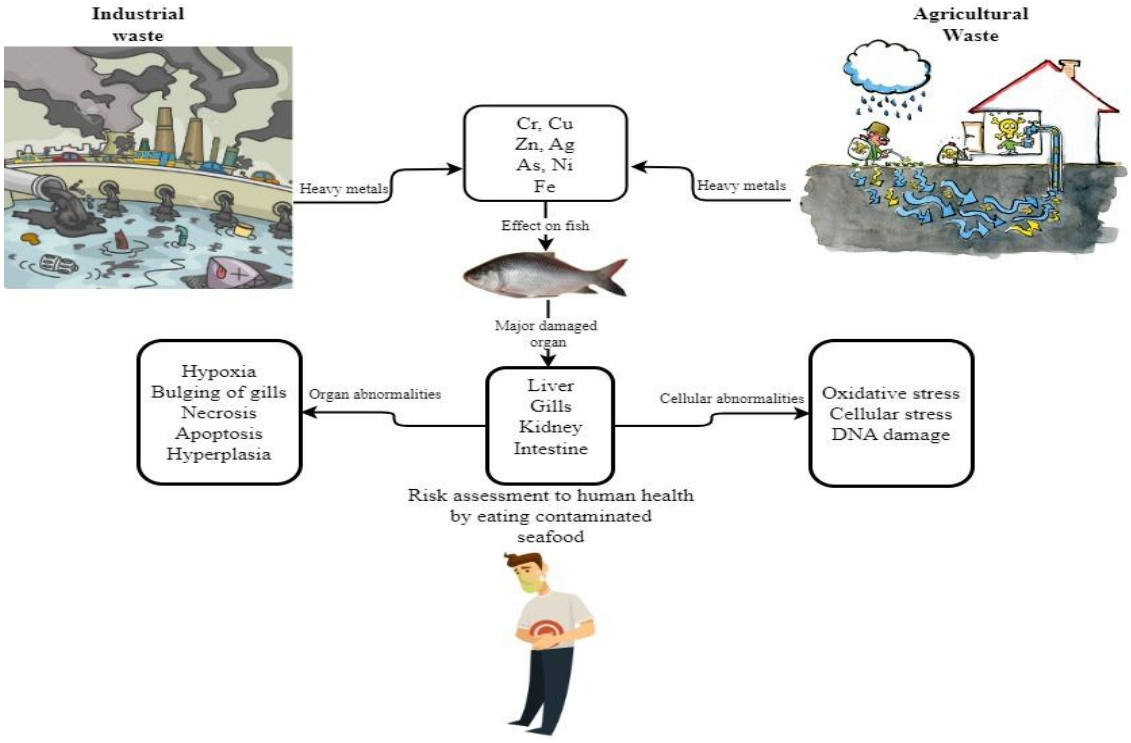
[1]Department of Zoology, The Islamia University of Bahawalpur, Pakistan.
[2]Department of Botany, The Government Sadiq College Women University Bahawalpur, Pakistan.
*Corresponding Authors
mariasaee2122@gmail.com
dr.abdul.ghaffar@iub.edu.pk

Abstract

An aquatic ecosystem is significantly contaminated by the unrestricted release of heavy metals from agricultural and industrial waste. Enhancement of industries correlates with improper dumping of waste products, influencing water pollution via releasing elevated concentrations of heavy metals, including nickel (Ni), chromium (Cr), copper (Cu), silver (Ag), cadmium (Cd), zinc (Zn), and iron (Fe). Metals are the primary motive for causing a variety of anomalies in aquatic animals, including fish. Commercially, humans use freshwater fish as food in various regions of Asia. The excessive release of these metals causes toxicity in fish, which is unsafe for human consumption. Heavy metals cause histological alteration and abnormalities in various fish species, including Indian significant carps (Thala, rohu, mori). Additionally, their toxicity causes oxidative stress in aquatic species. Various techniques have recently been applied to reduce toxic contaminants in marine environments. However, the bioremediation process plays a major role in lowering the toxicants through microorganisms, especially various species of bacteria. The objective of the recent study was to specify the specific organs (liver, gills, kidney, brain, muscle, and heart) of fish that are examined for histopathological impacts.

Keywords: Heavy-metals, Histopathology, oxidative stress, Indian major carps

Graphical Abstract



1. Introduction

In many previous years, the environmental pollution caused by various pollutants, including herbicides, pesticides, insecticides, and industrial waste, has become a major global issue. The release of several chemicals from such resources affects aquatic species by polluting their environment [1, 2]. The advances in industries related to nanotechnology continue; improper disposal of nanoparticles could introduce a new form of pollution into the aquatic environment, creating a viable threat to aquatic fauna [3]. The release of several environmental pollutants both directly and indirectly causes the excessive production of reactive oxygen species (ROS) in organisms [4], resulting in oxidative stress [1] and reduced antioxidant enzyme activity, leads damaging effects on various cell organelles, including protein, lipids, and DNA biomolecules [5]. Several researchers have demonstrated that alterations in fish morphology, oxidative stress, and antioxidant enzyme activity serve as valuable tools for early recognition of the toxicity of synthetic chemicals in birds [6] and other aquatic species, including fish [7]. The food chain and gills are precisely correlated for the contamination of heavy metals in marine life. These contaminated metals originate from oxidative stress, causing genotoxicity [8]. Various industries' excessive use of toxic heavy metals is causing significant damage to aquatic ecosystems. Consequently, these toxic heavy metals have accumulated in essential organs of fish within this contaminated environment, disrupting their normal functions [9]. Fish is preferred for ecotoxicological research because it can accumulate metal from sediments and water. Additionally, it is easily available and readily adapted to laboratory physiochemical parameters [10].

Researchers have introduced biomarkers as an excellent tool to recognize the effect of heavy metals on the aquatic environment. Among various fish species, major carp (*Labeo rohita)* is used as a biomarker due to its higher capacity for accumulation of metals, including Zn, Cd, Cr, Pb, Fe, and Ag, which help researchers to recognize the environmental effects of these toxins [11]. The accumulation of copper in fish gills shows a consistent rise in both the time and concentration of copper exposure, which increased significantly, with a clear dependence on time and dosage. This resulted in a substantial accumulation of copper in fish gills [3]. Fish is considered a rich source of protein, fats, zinc, iron, vitamins, minerals, and calcium for human energy requirements. Due to poor farming and water pollution, various infections have led to fish [12]. Water resources in Pakistan are polluted due to the discharge of a significant number of heavy metals from industrial waste without proper disposal, which causes water pollution [13]. Organisms' cellular structures are harmed by oxidative stress [1]. The metabolic liaises, catalase (CAT), superoxide dismutase (SOD), and total antioxidant/oxidant capability (TAS/TOS) have all been serve as indicators of oxidative stress in recent research. [14].

Our review aimed to estimate the toxicity of different metals, including Zn, Cu, Pb, Cd, Ni, Ag, and Fe, in aquatic environments mainly caused by free radicals. To better understand the mechanism of antioxidant defense caused by heavy metals, the current study investigates Peroxidase, Catalase, Glutathione, and Superoxide dismutase in fish liver, kidney, heart, brain, and gills. This makes it possible to analyze the concentration-dependent activity of antioxidant enzymes in these tissues. For this purpose, various bioremediation techniques were applied to reduce harmful concentrations of heavy metals from aquatic environments, including microbial bioremediation and Phyto-remediation. All such methods involved either the breakdown of toxic substances into less toxic substances or the complete degradation of such metals.

2. Materials and Methods

The current study was investigated to review the articles from 2015-2023 based on several histopathological changes in major carps. The following keywords such as aquaculture, heavy metals, histopathology, toxicity, fish species, and oxidative stress were used to search paper from PubMed and Google scholar. All included articles were cited along with their specific references.

2.1. Physiochemical analysis of water and sediments

In aquatic systems, toxic metals archetypally accumulate in

sediments or aquatic organisms. All waste products, among heavy metals, are considered the most hazardous on a universal scale due to their particularly toxic nature [15].In developing countries, water quality reflects their social, economic, and physiological aspects. However, these factors can cause the deterioration of water quality [16]. The acid digestion method calculates the number of toxic metals in the water sample [17]. Earlier studies suggested that the concentration of all metals in water increased in both post and pre-monsoon seasons. However, the concentration of lead (Pb) is still higher than all metals. In comparison, the chromium (Cr) concentration stays higher than other metals when samples are collected from sediments. Metals concentration in sediments becomes higher in summer than winter [18].To gain a detailed understanding of biochemical and physiological effects, it is necessary to compare fish's long and short-term exposure to direct toxicity [3].

2.2. Industrial Waste

The contamination of aquatic ecosystems is a global concern due to its impacts on water quality and seafood. The eminent level of these metals could be attributed to the release from steel and iron industries, the presence of dyes used in currency printing, and raw waste originating from native sources [16]. These metals can disrupt fish's physiological and biochemical mechanisms, causing harmful effects [19]. Metals like zinc, lead, copper, chromium, iron, arsenic, and cadmium are hypothetically harmful pollutants due to their ability to induce toxicity in fish [20].

2.3. Accumulation of Heavy metals in Aquatic Species

Fish hardly survive in poisonous water because heavy metals accumulate in various tissues, including gills, liver, kidney, muscle, and skin [9]. Heavy metals are deposited in fish's hard and soft tissues through bioaccumulation. Their accumulation contributes to figuring out the intensity of such heavy metals in the water environment. Heavy metals can be transferred from fish toward their predators through the food chain [21]. Some metals, including Zn, As, Cu, Cr, and Ni, have redox potential and undergo reactions to form reactive oxygen species (ROS) [22]. Gills are the primary source of bioaccumulation of heavy metals as they consistently contact the surrounding environment. Due to their thin and delicate structure, toxins and heavy metals are absorbed and dissolved in respiratory gases through gills [16]. They are highly susceptible to the effects of aquatic iron as it covers the gill surface and causes cellular destruction, leading to respirational abnormalities. The liver is a vital organ for iron storage. Therefore, excess iron has been stored in abundant fish species' livers [23].

2.4. Determination of heavy metals

Contamination resulting from heavy metals has become a critical problem for aquatic ecosystems worldwide because they are non-biodegradable and bioaccumulated in aquatic organisms. Deposition of heavy metals in fish tissues acts as an indicator of different toxicological effects [3]. From previous research, it was expected that heavy metals (i.e., Cr, Ag, Cd, Zn, and Ni, etc.) in higher concentrations accumulate in the different organs of fish through polluted water and transfer into the aquatic food chain. Due to an imbalance in the biogeochemical cycle, chromium enters the aquatic ecosystem through industrial waste, textile, mining, dyeing, and printing industries. Accumulation of chromium in *Labeo rohita* appears to have carcinogenic, genotoxic, poisonous, and mutagenic effects. A significant concentration of Cu can accumulate in the gills of fish. No mortar concentrations died at a lower concentration, but once the concentration exceeds, the mortality rate of fish flatters adversely [3, 24].

2.5. Arsenic

The World Health Organization has categorized arsenic as one of the most hazardous compounds to public health [25]. Arsenic, as a heavy metal, significantly impacts fish, affecting their physiology, growth, histopathology, and behavior [26]. When its concentration increased, there was a drop in the total weight of the fish. Many histopathological modifications were seen in the gills and intestines of fish exposed to such metal. At higher arsenic concentrations, significant damage was observed, like necrosis and infection in secondary lamellae of the gills [27]. Arsenic, as a heavy metal pollutant, exerts substantial adverse impacts on fish performance, growth, physiology,

histopathological conditions, and gene expression patterns [27].

2.6. Nickel

Previous observation estimated that Ni accumulates in the gills of fish and causes respiratory disorders [28]. Additionally, nickel shows a high to medium risk for public health and has cancer-causing and clastogenic lethal effects. It can produce more impact on Children than adults, and its inhalation led to several health issues, including pneumonia, edema, and even death [29, 30].

2.7. Copper

The higher concentration of copper causes inflammation of detoxifying organs and affects the reproductive organs of fish by reducing their fertility and hatching rate. [3, 31]. The primary harmful metal that contaminates the aquatic ecosystem is copper (Cu), which significantly affects the physiology and evolution of fish [32]. The most effective form of copper is copper-oxide nanoparticles (CuO-NPs), which can be used in agriculture, textile industries, coating, and drug delivery. In this form, copper bioaccumulates in the aquatic ecosystem and gets into the food chain, resulting in severe poisoning to non-target organisms [33, 34]. The introduction of sublethal concentration of CuO-NPs causes modifications in ecotoxicological factors like oxidative stress, metal accumulation, and genotoxicity after prolonged exposure [3].

2.8. Cadmium

Accumulation of Cd within the food chain is addicted to shrinkage of lobules, lowering motility, creating fibrosis, and viability of fish sperm [9]. A higher concentration of Cd in the Orontes River originates from industrial waste and can be transferred through the food chain and cause toxico-genetic effects in aquatic organisms [17]. When *Labeo rohita* was exposed to excessive Cd, it resulted in immunocompromised and susceptibility to disease [35].

2.9. Iron

Many organisms need iron for their physiological activities, but in higher concentrations, it causes toxicity. In mountainous states, the higher production of iron becomes a significant component that handles the abnormal productivity of aquatic organisms. Iron in the form of ferrous (Fe2+) was more toxic to aquatic species because it oxidized into ferric (Fe3+) iron, which binds gills and causes breathing dysfunction [23]. Further studies estimated that excess concentration of iron in aquatic systems causes DNA damage, oxidation of protein, and lipid peroxidation [17].

2.10. Silver

The high demand for silver production and consumption in industries and agriculture causes the accumulation of silver in the water ecosystem through their waste. The present study suggested that the ecotoxicological effect of silver on freshwater fishes causes DNA and molecular damage in commercial fish *Labeo rohita* [36]. When rainbow trout interact with silver-nano particles, they accumulate in the liver and gills of fish, starting oxidative stress. Ag-NPs cause kidney toxicity by renal alteration, cardiovascular collapse, and gills' ability to carry oxygen [37].

2.11. Chromium

Previous studies suggest chromium is one of the most essential elements used in over 50 industries [38]. Geographically, chromium is not found in its pure metallic form but exists in various oxidation states, including Cr2+ (divalent), Cr3+ (trivalent), and Cr6+ (hexavalent). The trivalent (Cr3+) and hexavalent (Cr6+) states are the most constant and widespread in natural environments [39]. Unnecessary accumulation of hexavalent chromium in aquatic organisms causes alteration in the physiology, histology, behavior, and morphology of fish. Acute exposure to Cr is highly toxic, which causes various health effects like DNA damage, decreased growth, reduction in protein level, decreased larval development, and erosion of fin-ray morphology [38].

2.12. Histological alteration and Abnormalities

The introduction of different insecticides and synthetic chemicals has detrimental effects on aquatic organisms, mainly fish, damaging their biodiversity and causing harm to the food chain. Histopathological studies of tissue evidence are a valuable method for assessing ecological pollutants' effect on essential fish organs within controlled laboratory situations [40].

Table 1. Heavy metals cause Histopathological Alterations in major carps.

Fish Species	Metal	Exposure Type &Time	Concentration	Histopathological irregularities	References
Labeo rohita	Arsenic	Lethal/ 14 days	15, 30, 35, 40, 45, 50, 55, 70 and 150 mg/L	Epithelial lifting, sever damage in the secondary lamellae of gills, necrosis, irregular shape, and damages in the gill raker were also observed in the primary lamellae of the gills.	[27]
Cirrhinus mrigala	Copper	Lethal/ 96 hours	7.21 mg/L	Mortality was recorded after each 24 hours for exposed fish.	[44]
Cirrhinus mrigala		Sub-lethal 30 days	1/10 of 96 h LC50	Erratic swimming, secretion of excess mucus, increase in surface breathing, necrotic lesions hyperplasia and restlessness were observed.	
Labeo rohita		Sub-lethal 30 days	1.34ppm	It can induce the OH radicals that can cause DNA damage, oxidation of bases and lipid peroxidation. Increased mortality rates of fish.	[45]
Cirrhinus mrigala		Sub-lethal 30 days	1.52 ppm	Mortality was observed.	
Labeo rohita		Sub-lethal 45 days	70.79 & 117.99 mg/L	Highest ratio of injured nuclei and genetic damage index was recorded in the fish erythrocytes cause oxidative stress, and genotoxicity in exposed fish.	[3]
Labeo rohita		Lethal 96 hours	353.98mg/L	Oxidative stress and genotoxicity were observed.	
Labeo rohita	Iron	Sub-lethal 96 hours	8.25, 16.51 and 33.01 mg L^{-1}	Increased number of red blood cells in fish which need higher oxygen demand. Damage of gill lamellae and hyperplasia causes hypoxia. Increased in WBC, tissue degeneration and Vocalization disintegration of cell boundaries.	[23]
Catla catla		Sub-lethal 150 days	40.05 mg L^{-1}	Reduced growth rate, histological alteration, disturbed feed intake, FCR, and fish physiology	[46]
Labeo rohita	Silver	Sub-lethal 7 days	100, 200, 400, and 800 mg l^{-1}	DNA damage, liver vacuolar degeneration, and hepatocytes degeneration was observed.	[36]
Labeo rohita		Sub-lethal 56 days	10, 15, and 20 µg kg^{-1}	shrunk gills filaments, loss of secondary lamella, tubular degeneration, interstitial necrosis, hemorrhages, and interstitial mononuclear cell infiltration were observed.	[37]
Labeo rohita	Chromium	Sub-lethal 28 days	37, 22, and 11 mgL^{-1}	Histological changes were observed in gills, kidney, and liver such as , lamellar curling, oedema secondary lamellae fusion, telangiectasia, and hyperplasia.	[47]
Labeo rohita	Cadmium	Sub-lethal 28 days	0.65 mg CdCl2 L-1	Fish observation reduced enzymatic and phagocytic activity displaying Immunosuppressive directed to disease susceptibility.	[35]
Catla catla		Sub-lethal 96 hours	0.4225 mg L-1	Histological changes such as hemorrhage, necrosis, curling, oedema, and hyperplasia.	[48]
Labeo rohita	Zinc	Lethal 96 h	31.15 mg/L	Oxidative stress can lead to mortality of fish.	

					[49]
Labeo rohita		Sub-lethal 80 days	1/3rd of 96-h LC50	Neutralized CAT and SOD activity, leading to increased reactive oxygen species (ROS) generation in fish.	
Catla catla	Nickel	Sub-lethal 60 days	7.40 mg L-1	Increased in weight, total length, and fork length	[50]

Heavy metals result in physiological and reproductive abnormalities in fish [41]. In addition, pathological marker enzymes were assessed to evaluate the liver function of *Labeo rohita*, a primarily herbivorous fish that predominantly consumes vegetation and phytoplankton [16]. Accumulation of such metals resulted in a significant decline in energy reserves, including protein, glycogen, and glucose, and induced alteration in hematological parameters. Additionally, liver function tests discovered damage to fish liver due to exposure [16]. Several researchers investigate metals' histological effects on major fish organs, including the liver, kidney, intestine, and gills [42]. Once a fish is exposed to sub-lethal and lethal concentrations of such contaminants results in deformed physiology, which affects the nervous, GI, and respiratory systems [43]. Metals-exposed fish indicated hepatocyte swelling in the liver and hyperplasia in gills lamellae, as studied by [23].

3. Biomarkers of Heavy metals toxicity

Many biochemical and physiological conditions are indicated with the help of several biomarkers which serve as interface between agent of aquatic ecosystem and body of fish [11, 38].When a fish (*Labeo rohita*) exposed to 39.4 mg/1 of hexavalent chromium, then stress protein (metallothionine) serves as biomarker which can decrease the total concentration of protein, lipids and glycogen in gills ,muscles and liver tissues [38].Biomarkers have ability to show the relationship between heavy metal exposure and biological modification in organisms. However, many biomarkers have been studied to exhibit the biological alteration in aquatic organisms in both natural and experimental environments due to introduction of various chemicals [11].The hematological changes in fish body such as decreased RBC and hemoglobin percentage are considered as biomarkers [51].

4. Antioxidant Enzymes

The biomarkers of enzymes are used to detect the physical changes in target tissue caused by heavy metals such as Cr, Cu, As, and Pb, which may disturb the structural activities of enzymes in aquatic ecosystems [11]. Numerous studies reported that antioxidant enzyme biomarkers are reliable tools for extracting and screening the toxicity of metals in marine organisms like fish. However, various antioxidant enzymes include catalase, superoxide dismutase, and peroxidase, which can reduce the ROS [6]. The existence of stressors has been providing evidence to elevate oxidative stress levels, as determined through a capacity of catalase, SOD, and GST activity [52]. The activity of SOD and CAT enzymes in both the gills and liver of fish exposed to iron was notably lower when compared to the control group. Furthermore, these enzyme activities are dosage-dependent and consistently decrease as the test concentration and exposure time increase [23].

4.1. Catalase (CAT)

Several investigations suggested that catalase enzymes play a significant role in oxidative stress and have higher activity in the liver, erythrocytes, and kidneys. Catalase is a helpful biomarker for liver damage caused by pesticide accumulation [53, 54]. The method of Aebi (1984) was based on its ability to decrease the H2O2 concentration at 240 nm at 15-second pauses for a total of 90 seconds. For this purpose, tissue homogenates were treated with a familiar inhibitor of catalase activity (sodium azide) [15, 55]. Therefore, analyzing the catalase activity of *Labeo rohita* liver from a farmed habitat

displayed the highest enzyme activity, while the gills of hatchery fish exhibited the slightest enzyme activity [56].

4.2. Super-oxide dismutase (SOD)

The activity of SOD was measured by using the spectrophotometric method depending on the evaluation of $O2^{-}$-mediated nitro blue tetrazolium reduction in the presence of an aerobic mixture of NADH and phenazine methosulfate [55]. The SOD level in the liver of *Labeo rohita* measured antioxidant enzyme suppression when fish were exposed to various concentrations of heavy metal. Elevation in heavy metal concentration led to decreased SOD after 96 hours [57]. The order of SOD activity in the significant organs of fish is shown as liver>gills>kidney [15].

4.3. Glutathione S-transferase (GST)

The GST activity was measured in a spectrophotometric manner. The same response was observed in the mullet but not in Nile tilapia, which increased the antioxidant defense of fish collected from the contaminated region. GST emphasizes the requirement for hepatocytes to deplete peroxides in the liver. Frequently, it is affected by various types of organic and inorganic pollutants after contact with them [55].

5. Oxidative stress

Fish exposed to heavy metals produce mitochondrial abnormalities, DNA damage, and oxidative stress. These findings contrast with those of our research. A significant source of heavy metal toxicity that can cause a fish physiological imbalance is respiratory discomfort [58]. Oxidative stress occurs when the balance between antioxidant enzymes and reactive oxygen species (ROS) is disturbed, leading to histopathological destruction. This can result from increased ROS and the inability to repair oxidative damage. Aquatic organisms sustain redox homeostasis through their antioxidant enzyme defense system. Oxidative stress can harm tissues by oxidating protein and DNA and peroxidating unsaturated lipids [15]. The oxidative stress marker is essential for damaging DNA, cell membranes, and proteins. [11, 17].

Figure 1. Heavy metals exposure causes oxidative stress in fish.

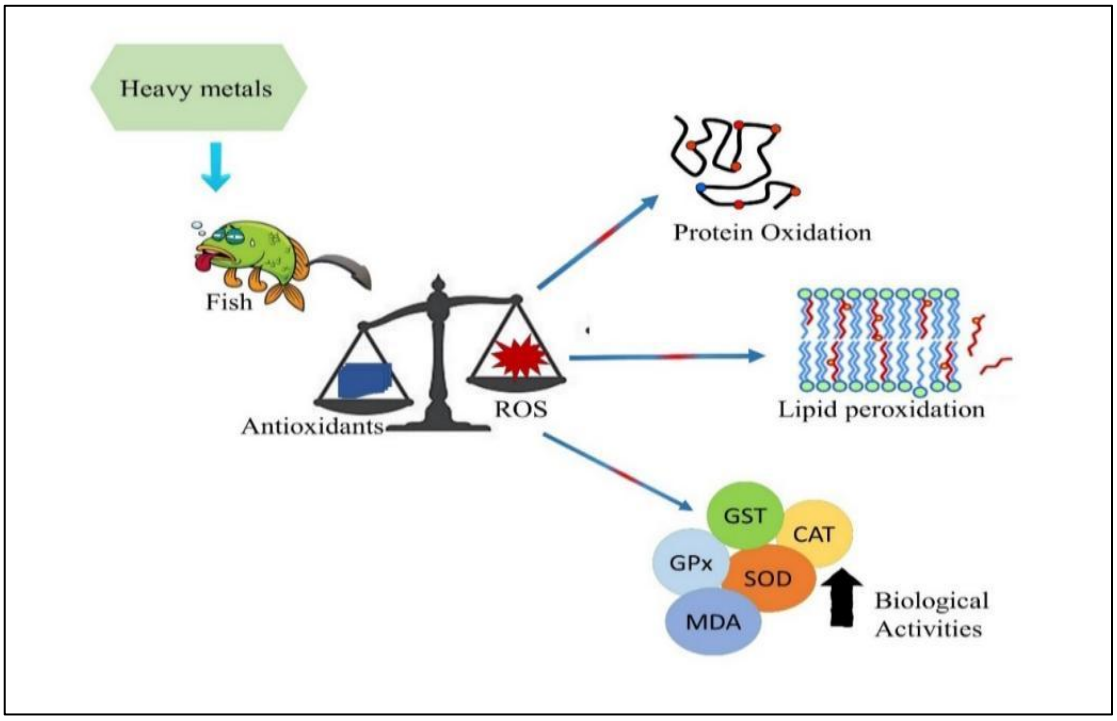


Table 2. The impacts of heavy metals on antioxidant enzymes activity in various organs of major Carps.

Fish	Metal	Target	Increased	Decreased	Antioxidant-enzyme	References

species		organ			Abnormalities	
Labeo rohita	Fe, Mn, Zn, Cu, Ni, Cr, and Cd	Gills Liver	SOD LPO	CAT GSH GST	Oxidative stress and DNA damage.	[11]
Cirrhinus mrigala	Cu and Cd	Liver Kidney Gills	SOD	LPO CAT	Reduced growth and increased oxidative stress in exposed fish.	[15]
Labeo rohita	Zn, Cu, and Ni	Liver Kidney Gills	SOD LPO		Higher α- amylase activity was found in liver of hatchery fish and lower in kidney of farmed fish. Result in oxidative stress	[56]
Labeo rohita	ZnO-NPs	Muscles Liver Gills Heart		SOD	Increased the production of ROS (reactive oxygen species) in fish and the level of TBARS increased the risk of oxidative stress.	[49]
Labeo rohita	Cr, Cu, and Cd	Liver	LPO	GSH SOD	Significantly increase in ROS, referred to oxidative stress led to DNA damage.	[57]
Catla catla	cu	Gills Liver Kidney Muscles Brain	SOD POD	CAT	Increased ROS level caused intensified DNA damage and oxidative stress.	[61]
Labeo rohita	As	Brain Gills	LPO	CAT GSH GPx	Elevation of reactive oxygen species (ROS) causes oxidative stress.	[62]
Cirrhinus mrigala	Cd	Gills Liver Kidney	LPO	GSH GPx GST	Increased reactive oxygen species (ROS) which generate oxidative stress and free radicals.	[63]
Labeo rohita	CdCl2	Gills	LPO	CAT GSH GPx GST	Production of ROS cause oxidative stress and DNA damage.	[64]
Catla catla	Pb^{+2}	Brain Liver Gills Kidney Muscle Heart	POD		Oxidative stress	[65]

The evaluation of oxidative stress in fish gills is involved in the measurement of catalase (CAT) activity and the level of thiobarbituric acid reactive substance (TBARS) [3]. Reactive oxygen species (ROS) are essential components in immune system adjustment, redox stability, and the development of cellular signaling systems. Unnecessary formation of ROS can destroy cellular lipids, proteins, nucleic acids, membranes, and organelles, ultimately causing apoptosis [59].

5.1. Genotoxicity

Exposure of *Labeo rohita* to various concentrations of copper oxide nanoparticles causes damage to nuclei and genetic abnormalities. These two metrics show a consistent increase in

fish tissues with the rise in concentration and exposure time [3]. The inability of fish to repair DNA due to oxidative stress causes genomic instability [58]. Excessive release of ROS causes degeneration of antioxidant enzymes, oxidative stress, and injury to various cells of organelles like protein, lipids, and DNA damage [60].

6. Risk Assessments to Human Health

Heavy metals like Al, As, Cr, and Pb accumulate in various species and cause severe damage to fish organs. Such contaminants pose potential hazards to consumers by consuming the polluted fish. Introducing heavy metals and pollutants into the food chain through various paths poses a significant danger to human health. Due to carcinogenic and noncarcinogenic health risk assessments, the major carp is not suitable for human consumption [26]. Bioaccumulation of Chromium causes DNA damage in the muscle cells of fish that could be consumed as food by humans and become harmful to their health [66]. Both cadmium (Cd) and lead (Pb) are included in harmful metals of the European Union that bioaccumulate in fish and contaminate seafood. These contaminated fish are highly toxic to humans, even when they are in very little concentration when they consume contaminated seafood [18].

6.1. Bioremediation process

In bioremediation procedures, several microorganisms are utilized to eliminate heavy metals from the environment [9]. The bioremediation method is more effective when environmental conditions allow microorganisms to grow because it depends upon naturally occurring microbes in soil and poses no harm to people living in that environment [67].

6.2. Microbial bioremediation

Fungi, bacteria, and algae are the microorganisms needed [68]. A bacterial mixture of isolated strains used in the bioremediation method effectively removes heavy metals from aquatic environments. These strains include *Sporosarcina soil* B-22, *Viridibacillus arenose* B-21, and *Enterobacter cloacae* KJ-46 and KJ-47[69]. In the biosorption and precipitation process, Bacillus is employed to reduce heavy metals from aquatic environments [70]. Phytoremediation methods use a variety of plants and bacteria to remove heavy metals and maintain a clean environment [71].

7. Conclusions and Recommendations

The current review indicates that exposure to various heavy metals, including Ni, Cr, Cu, Ag, Cd, Zn, and Fe at higher concentrations, cause histopathological changes and oxidative stress in Indian major carps (Thala, rohu, and Mori). However, such histopathological changes would play a key role in observing the effects of heavy metals on fish species. Oxidative stress is attributed to two main factors: the production of ROS and the reduced enzyme antioxidant activity. Hence, the present study served as a tool to estimate the water pollution caused by heavy metals.

Data Availability statement

The data used to support the outcomes of this study is available from the corresponding author on request.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors Contribution

All authors participated in the initial draft creation, reviewed the manuscript, and contributed to the editing process.

Acknowledgment

The authors feel grateful to Prof. Dr. Abdul Ghaffar (The Islamia University of Bahawalpur) for his technical support and guidance.

Funding

No funds were provided by any national or international funding agencies.

REFERENCES

1. Akram, R., et al., Hematological, serum biochemistry, histopathological and mutagenic impacts of triclosan on fish (bighead carp). Agrobiological records, 2022. **7**: p. 18-28.
2. Ahmed, H., et al., Efficacy of melatonin against oxidative stress, DNA damage and histopathological changes induced by nicotine in liver and kidneys of male rats. International journal of veterinary science, 2021. **10**(1): p. 31-36.
3. Aziz, S. and S. Abdullah, Evaluation of Toxicity Induced by Engineered CuO Nanoparticles in Freshwater Fish,

Labeo rohita. Turkish Journal of Fisheries and Aquatic Sciences, 2023. **23**(8).

4. Hussain, R., et al., Thiamethoxam at sublethal concentrations induces histopathological, serum biochemical alterations and DNA damage in fish (Labeo rohita). Toxin Reviews, 2022. **41**(1): p. 154-164.
5. M Hussein, N., et al., Ameliorative role of chitosan nanoparticles against bisphenol-A induced behavioral, biochemical changes and nephrotoxicity in the African catfish, Clarias gariepinus. Egyptian Journal of Aquatic Biology and Fisheries, 2021. **25**(1): p. 493-510.
6. Merdana, I., et al., The effect of ethanolic extract of Myrmecodia pendans on gentamicin induced nephrotoxicity in Wistar rats. International journal of veterinary science, 2021. **10**(2): p. 96-101.
7. Ghaffar, A., et al., Assessment of genotoxic and pathologic potentials of fipronil insecticide in Labeo rohita (Hamilton, 1822). Toxin Reviews, 2021. **40**(4): p. 1289-1300.
8. Bibi, S., et al., A review on histopathological alterations induced by heavy metals (Cd, Ni, Cr, Hg) in different fish species. Punjab Univ J Zool, 2021. **36**: p. 81-89.
9. Jamil Emon, F., et al., Bioaccumulation and Bioremediation of Heavy Metals in Fishes—A Review. Toxics, 2023. **11**(6): p. 510.
10. Naguib, M., et al., Genotoxic evaluation of silver nanoparticles in catfish Clarias gariepinus erythrocytes; DNA strand breakage using comet assay. Scientific African, 2022. **16**: p. e01260.
11. Mahamood, M., et al., Labeo rohita, a bioindicator for water quality and associated biomarkers of heavy metal toxicity. NPJ Clean Water, 2021. **4**(1): p. 17.
12. Ullah, I., et al., Encouraging knowledge sharing behavior through team innovation climate, altruistic intention and organizational culture. Knowledge Management & E-Learning, 2016. **8**(4): p. 628.
13. Bandpei, A.M., et al., Bioaccumulation of heavy metals muscle of common carp fish (Cyprinus carpio L, 1758) from Ala gul and Alma gul wetlands of Golestan and consumption risk assessment. International Journal of Medical Research & Health Sciences, 2016. **5**(11): p. 267-273.
14. Nur, F., et al., Diversity, distribution, and conservation status of Betta fish (Teleostei: Osphronemidae) in Aceh Waters, Indonesia. The European Zoological Journal, 2022. **89**(1): p. 142-151.
15. Hassan, W., et al., The effect of sub-lethal level of dietary copper and cadmium on the growth performance and oxidative stress in Cirrhina mrigala. Pak J Agric Sci, 2015. **52**: p. 789-794.
16. Tabrez, S., T.A. Zughaibi, and M. Javed, Water quality index, Labeo rohita, and Eichhornia crassipes: Suitable bio-indicators of river water pollution. Saudi Journal of Biological Sciences, 2022. **29**(1): p. 75-82.
17. Turan, F., et al., Heavy metal bioaccumulation, oxidative stress and genotoxicity in African catfish Clarias gariepinus from Orontes river. Ecotoxicology, 2020. **29**: p. 1522-1537.
18. Rasib, K., Z. Ejaz, and D. Hussain, Toxicity of heavy metals in water, sediments and different body organs of fish Labeo rohita (rohu) at head Balloki, river Ravi, Lahore Pakistan. 2022.
19. Shaukat, N., et al., Oxidative stress biomarker in assessing the lead induced toxicity in commercially important fish, Labeo rohita. Pakistan Journal of Zoology, 2018. **50**(2).
20. Latif, F. and M. Javed, Cadmium Induced Oxidative Stress in Different Organs of Labeo rohita and Catla catla. 2018.
21. Valkova, E., et al., Content of Pb and Zn in Sediments and Hydrobionts as Ecological Markers for Pollution Assessment of Freshwater Objects in Bulgaria—A Review. International journal of environmental research and public health, 2022. **19**(15): p. 9600.
22. Yu, B., et al., Heavy metal concentrations in aquatic organisms (fishes, shrimp and crabs) and health risk assessment in China. Marine Pollution Bulletin, 2020. **159**: p. 111505.

23. Singh, M., et al., Iron mediated hematological, oxidative and histological alterations in freshwater fish Labeo rohita. Ecotoxicology and environmental safety, 2019. **170**: p. 87-97.
24. Dhara, K., et al., Effects of short-term selenium exposure on respiratory activity and proximate body composition of early-life stages of Catla catla, Labeo rohita and Cirrhinus mrigala. Environmental toxicology and pharmacology, 2022. **90**: p. 103805.
25. Babich, R. and R.J. Van Beneden, Effect of arsenic exposure on early eye development in zebrafish (Danio rerio). Journal of Applied Toxicology, 2019. **39**(6): p. 824-831.
26. Naz, S., et al., Bioaccumulation Pattern and Health Risk Assessment of Heavy Metals in Cirrhinus mrigala at Panjnad Headworks, Bahawalpur, Pakistan. Toxics, 2023. **11**(7): p. 596.
27. Rabbane, M.G., et al., Toxic effects of arsenic in commercially important fish Rohu Carp, Labeo rohita of Bangladesh. Fishes, 2022. **7**(5): p. 217.
28. Blewett, T.A., C.M. Wood, and C.N. Glover, Salinity-dependent nickel accumulation and effects on respiration, ion regulation and oxidative stress in the galaxiid fish, Galaxias maculatus. Environmental Pollution, 2016. **214**: p. 132-141.
29. Rani, I., Studies on Bioabsorption ability of Algae and fungi inhabiting coast of Karachi. 2017, University of Karachi, Karachi.
30. Mehnaz, M., et al., Prediction of Hazardous Effect of Heavy Metals of Point-Source Wastewater on Fish (Anabas cobojius) and Human Health. Biological Trace Element Research, 2023. **201**(6): p. 3031-3049.
31. Forouhar Vajargah, M., et al., Effects of copper oxide nanoparticles (CuO-NPs) on parturition time, survival rate and reproductive success of guppy fish, Poecilia reticulata. Journal of cluster science, 2020. **31**: p. 499-506.
32. Mitra, S., et al., Dissolved trace elements in Hooghly (Ganges) River Estuary, India: Risk assessment and implications for management. Marine pollution bulletin, 2018. **133**: p. 402-414.
33. Prasad, P.R., S. Kanchi, and E. Naidoo, In-vitro evaluation of copper nanoparticles cytotoxicity on prostate cancer cell lines and their antioxidant, sensing and catalytic activity: One-pot green approach. Journal of Photochemistry and Photobiology B: Biology, 2016. **161**: p. 375-382.
34. Keller, A.A., et al., Comparative environmental fate and toxicity of copper nanomaterials. NanoImpact, 2017. **7**: p. 28-40.
35. Giri, S.S., et al., Immunotoxicological effects of cadmium on Labeo rohita, with emphasis on the expression of HSP genes. Fish & shellfish immunology, 2016. **54**: p. 164-171.
36. Sharma, N., et al., Assessment of DNA damage and molecular responses in Labeo rohita (Hamilton, 1822) following short-term exposure to silver nanoparticles. Food and chemical toxicology, 2016. **96**: p. 122-132.
37. Popoola, O.M., B.K. Behera, and V. Kumar, Dietary silver nanoparticles as immunostimulant on rohu (Labeo rohita): Effects on the growth, cellular ultrastructure, immune-gene expression, and survival against Aeromonas hydrophila. Fish and Shellfish Immunology Reports, 2023. **4**: p. 100080.
38. Saleem, A., et al., Effect of Chromium Exposure on the Antioxidant Enzymes Activity and Haematological Indices in Labeo rohita. East African Scholars Multidiscip Bull, 2022. **5**(5): p. 94-102.
39. Deepak, T., G. Sanjay, and C. Shivakumar, Acute toxicity of hexavalent chromium on behavioural parameters and histopathology damages in freshwater common carp, Cyprinus carpio. Asian Journal of Research in Zoology, 2021. **4**(3): p. 1-8.
40. Javed, M. and N. 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, 2019. **89**: p. 389-403.
41. Naseem, S., et al., Inquisition of Toxic Effects of Pyriproxyfen on Physical, Hemato-Biochemical and

Histopathological Parameters in Labeo rohita Fish. Pakistan veterinary journal, 2022. **42**(3).

42. Padhy, G., C. Mishra, and B.P. Barik, Histological alterations in gill tissues of Anabas testiduneus on exposure to heavy metal CdCl2. RJLBPCS, 2018. **4**: p. 328-334.
43. Kumari, B., et al., Toxicology of arsenic in fish and aquatic systems. Environmental chemistry letters, 2017. **15**: p. 43-64.
44. Vanitha, S., et al., SUBLETHAL EFFECTS OF LEAD ARSENATE ON HISTOLOGY OF SELECTED ORGANS OF FRESHWATER FISH CIRRHINUS MRIGALA. International Journal of Zoology and Applied Biosciences, 2017. **2**(5): p. 250-257.
45. Afridi, A.J., et al., Hemp (Marijuana) reverted Copper-induced toxic effects on the essential fatty acid profile of Labeo rohita and Cirrhinus mrigala. Molecular biology reports, 2019. **46**: p. 391-401.
46. Hussain, S.M., et al., Growth responses of Catla catla, Labeo rohita and Cirrhina mrigala during chronic exposure of iron. Pakistan Journal of Agricultural Sciences, 2011. **48**(3): p. 225-230.
47. Chaudhary, A., K. gul Javaid, and E. Bughio, Toxic effects of chromium chloride on hematology and histopathology of major carp (Labeo rohita). The Egyptian Journal of Aquatic Research, 2023.
48. Hussan, A., et al., Effect of mercury and cadmium on the oxygen consumption and gill Histology of Catla catla (Ham. 1822). Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 2018. **88**: p. 729-738.
49. Aziz, S., et al., Effects of Engineered Zinc Oxide Nanoparticles on Freshwater Fish, Labeo rohita: Characterization of ZnO Nanoparticles, Acute Toxicity and Oxidative Stress. Pakistan Veterinary Journal, 2020. **40**(4).
50. Safina Kousar, M.J., M. Akhter, and S. Nadeem, Effect of dietary nickel concentrations on the growth and feed conversion efficiency of fish. 2020.
51. Khalid, M., et al., Heavy Metals (Copper, Chromium And Cadmium) Induced Oxidative Stress Biomarkers On Haematological Parameters Of Labeo Rohita. Oxidation Communications, 2016. **39**.
52. Ibrahim, D., et al., Dual effect of Selenium loaded Chitosan Nanoparticles on growth, antioxidant, immune related genes expression, transcriptomics modulation of caspase 1, cytochrome P450 and heat shock protein and Aeromonas hydrophila resistance of Nile Tilapia (Oreochromis niloticus). Fish & Shellfish Immunology, 2021. **110**: p. 91-99.
53. Glorieux, C., et al., Regulation of catalase expression in healthy and cancerous cells. Free Radical Biology and Medicine, 2015. **87**: p. 84-97.
54. Clasen, B., et al., Bioaccumulation and oxidative stress caused by pesticides in Cyprinus carpio reared in a rice-fish system. Science of the total environment, 2018. **626**: p. 737-743.
55. Das, D., et al., Effect of heavy metals on tissue-specific antioxidant response in Indian major carps. Environmental Science and Pollution Research, 2017. **24**: p. 18010-18024.
56. Anjum Zia, M., et al., Sublethal impacts of heavy metals on antioxidant enzymes and biochemical parameters in rohu (Labeo rohita). Iranian Journal of Fisheries Sciences, 2017. **17**(2): p. 668-683.
57. Khalid, M., et al., Heavy metals (chromium, copper and cadmium) induced oxidative stress in Labeo rohita (Hamilton, 1822) during acute and chronic toxicity experiment. Int. J. Biosci, 2015. **6**(11): p. 64-72.
58. Yamin, A., et al., Exposure to low concentrations of heavy metals alone and in combination induces histopathological and genotoxic effects in fish (Labeo rohita). Advancements in life sciences, 2020. **7**(4): p. 240-246.
59. Chowdhury, S. and S. Saikia, Oxidative Stress in Fish: A Review. Journal of Scientific Research, 2020. **12**(1).
60. Li, X., et al., Evaluation of DNA damage, biomarkers of oxidative stress, and status of antioxidant enzymes in

freshwater fish (Labeo rohita) exposed to pyriproxyfen. Oxidative Medicine and Cellular Longevity, 2022. **2022**.

61. Latif, F., et al., Studies on bioaccumulation patterns, biochemical and genotoxic effects of copper on freshwater fish, Catla catla: an in vivo analysis. Brazilian Journal of Biology, 2022. **84**: p. e256905.
62. Aruljothi, B. and S.S. Samipillai, Effect of arsenic on lipid peroxidation and antioxidants system in fresh water fish, Labeo rohita. Int. J. Mod. Res. Rev, 2014. **2**(5): p. 15-19.
63. Malarvizhi, A., et al., Accumulation of cadmium and antioxidant and hormonal responses in the Indian Major Carp Cirrhinus mrigala during acute and sublethal exposure. Water, Air, & Soil Pollution, 2017. **228**: p. 1-14.
64. Dabas, A., et al., Investigation of cadmium-induced genotoxicity and oxidative stress response in Indian major carp, Labeo rohita. Human and Ecological Risk Assessment: An International Journal, 2014. **20**(2): p. 510-526.
65. Nosheen, S.A., H. Naz, and K. Abbas, Toxicological effects of heavy metal (Pb+ 2) on peroxidase activity in freshwater fish, Catla catla. Pakistan Journal of Agricultural Research, 2019. **32**(4): p. 656-661.
66. Suchana, S.A., et al., Chromium exposure causes structural aberrations of erythrocytes, gills, liver, kidney, and genetic damage in striped catfish Pangasianodon hypophthalmus. Biological Trace Element Research, 2021. **199**: p. 3869-3885.
67. Medfu Tarekegn, M., F. Zewdu Salilih, and A.I. Ishetu, Microbes used as a tool for bioremediation of heavy metal from the environment. Cogent Food & Agriculture, 2020. **6**(1): p. 1783174.
68. Kim, I.H., et al., Development of a microbe-zeolite carrier for the effective elimination of heavy metals from seawater. Journal of microbiology and biotechnology, 2015. **25**(9): p. 1542-1546.
69. Kang, C.-H., Y.-J. Kwon, and J.-S. So, Bioremediation of heavy metals by using bacterial mixtures. Ecological engineering, 2016. **89**: p. 64-69.
70. Pham, V.H.T., et al., Bacterial biosorbents, an efficient heavy metals green clean-up strategy: Prospects, challenges, and opportunities. Microorganisms, 2022. **10**(3): p. 610.
71. Dixit, R., et al., Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability, 2015. **7**(2): p. 2189-2212.

How to cite this article: Khan MS, Ghaffar A, Jamil H, Khalid S, Tafazul B. Heavy metals cause Toxicity, Histopathological Abnormalities and Oxidative stress in major carps (*Catla catla, Labeo rohita and Cirrhinus mrigala*). *Journal of Zoology and Systematics*, 2(1), 10–22.