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Enzymatic Modifications, as Biomarker for Heavy Metal Toxicity in *Catla catla* a Fresh Water Fish

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Authors' contributions

This work was carried out in collaboration among all authors. All authors were involved for sampling, field work, laboratory activities, data collection and statistical analysis. The manuscript was prepared and edited by author AJ. All authors have read and approved the manuscript.

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ABSTRACT

Over the last couple of decades, pollution of the environment has grown to be a significant problem, attracting the curiosity of countless experts from developed as well as underdeveloped countries. Sewage dumping into waterways has a detrimental effect on the aquatic ecosystem and biota since it is the principal sink. As a result of their ability to bioaccumulate and intensify as they ascend food chains, toxic metals are not completely eliminated from the environment. For instance, heavy metals have a tendency to accumulate in the muscular tissues of aquatic species; hence, tissues with elevated levels of toxic metals may be detrimental to the well-being of humans and other animals. Fish have been used as indicator organisms to track contamination in aquatic environments. The fish known as *catla catla*, an Indian Major carp, is edible and extremely susceptible to even the slightest amount of stress. Fish biomarkers are now often employed to assess the internal and exterior health conditions brought on by toxins.

The purpose of our investigation was to clarify how biomarkers may be used to track and assess the levels of toxic heavy metals in freshwater *Catla catla* fish.

Results: SOD can be used as a bioindicator for heavy metal pollution, according to the current study's findings. These investigations ultimately aim to quantify pollution concentrations that can cause aquatic environments to undergo permanent biological alterations. Though still mild, the amount of toxicity was approaching to danger. To preserve a healthy and pristine environment, public and government-sponsored initiatives can be undertaken to restrict those activities that discharge contaminants into the ecosystem in an unscientific way.

Conclusion: The study comes to the conclusion that in order to evaluate and track the biological state of the aquatic environment, a multiparameter assessment is required.

*Keywords: Heavy metal toxicity; catla catla; biomarkers; antioxidant enzyme (*Superoxide *Dismutase); oxidative stress; Reactive oxygen species (ROS).*

1. INTRODUCTION

Fish are considered ideal test subjects in ecotoxicological studies because they serve as biomarker indicators to determine the hazardous effects of pollutants in waterways and are extensively utilized because they are inexpensive, readily accessible, amenable to experimental conditions, and demonstrate different levels of susceptibility to toxins. Fishes are vulnerable to ecological contamination in water bodies since they are among the most widely spread aquatic animals. They serve as indicators of contamination from the environment and can be utilized to assess the overall wellness of aquatic ecosystems.

Water bodies serve as significant reservoirs for commercial, residential, and other human made substances, [1]. Water contamination has widespread effects on the creatures residing in these environments. Fish, as inhabitants of water systems, are unavoidably affected by these harmful pollutants. There are a number of indicators that are frequently used to assess the biological effects of contaminants. These markers serve as initial warnings of particular harmful physiological impacts. Histopathological indicators and oxidative damage are used in environmental toxicology [2]. A trustworthy surveillance technique that enables the assessment of external stressor impacts is determining the histology of fish organs. It serves as one of most accurate markers of health deterioration brought on by stresses created by humans in aquatic life [3] Leonardi, Tarifeno, Vera, 2009).

In order to combat the effects of reactive oxygen species, animals have both enzymatic and non-enzymatic antioxidants as essential defences against atmospheric pro-oxidants [4]. As possible indicators, antioxidant measures and cellular oxidative stress indicators are commonly utilized as diagnostic methods to evaluate the effects of environmental damage. Catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferases (GST), and glutathione peroxidase (GPx) are significant antioxidant enzymes. Furthermore, antioxidant glutathione vitamins, and beta-carotene boost the defensive enzyme mechanisms in the body and help animals to reduce exposure to external toxins.

The term "biomarker" refers to assessments in bloodstream fluids, cells, and organs that reveal molecular or cellular alterations brought on by the existence and strength of toxins. They function as initial detection instruments for identifying biological impacts and assessing the condition of the environment. According to Mahboob *et al*. [5] and Sultana *et al*. [6] biomarkers are crucial for determining the possible physical and chemical hazards in ecosystems. For the purpose of observing aquatic habitats, aquatic creatures, notably fish, are frequently used. Due to their ability to gather, metabolise, concentrate, and accumulate toxins from the water sources, fish are excellent monitors of the effects of different chemicals dumped into water bodies. As a result, these conditions may cause fish to develop DNA damage [7,8]. Concerns over environmental issues, particularly water contamination, have grown around the world throughout the past three decades. According to Afshan *et al*. [9] Garg *et al*. [10] and Nagarani *et al*. [11] water is essential for the transmission of nutrients across all ecosystems, which eventually endangers aquatic creatures and leads to humans through the food chain. According to Pruß-Ustün *et al*. [12] prolonged contact with environmental contamination is the cause of around 25% of the illnesses that affect humans today. According to reports, the two greatest global issues are the disposal of trash in aquatic ecosystems and pollution Anh *et al*., [13] Arkoosh *et al*., [14] In all regions where they occur, environmental contaminants in gas, solid, and liquid states pose a risk to people and animals, either individually or in combination Kovacik, [15]. The accumulation of heavy metals harms human health in the long run because of the harmful effects of these chemicals on aquatic life, even if their typical occurrence is not hazardous to the ecosystem. According to Nagarajan *et al*. (2020) and Shuhaimi-Othman *et al*. [16] there is evidence that the presence of elevated pollution levels, namely heavy metals, can impede biological and physiological procedures that are essential for fish metabolism. According to Zhang *et al*. [17] the absorbed amount of any metallic substance that is beyond a certain threshold leads to permanent physiological responses. Even though there are many naturally occurring sources of toxic metals, human activities contribute more to heavy metal contamination than natural releases, particularly when it comes to Pb, Hg, Cd, Zn,Cr,Cu and Ni. This is evident in reports from the past that discuss anthropomorphic and industrial discharges that enter freshwater and marine ecosystems Bhattacharyya *et al*., [18]. Through the formation of metallic complexes, toxic metals impede the activities of structural amino acids, digestive enzymes, and DNA [19]. Furthermore, it also causes genomic abnormalities, structural or physiological modifications, and immunological system dysfunction [20]. The toxicology of heavy metals in fish is largely determined by a number of physico-chemical factors, including the element's solubility, hardness, pH, and ecosystem complexity as expressed through the skin, diet, and gills [21]. Fish constitutes one of the primary food sources for people. Additionally, because this fish occupies the top of the aqueous food chain, it can serve as one of the finest biosensors of aquatic contamination. Fish may absorb and collect metals from the water around them directly or, subsequently, through other animals, including tiny fish, crustaceans, and aquatic plants. Fish at the top of the aquatic food chain tend to store contaminants in their fatty organs, such as the liver. They can also accumulate metallic substances, which they can then pass on to humans through food, leading to either acute or long-term illnesses [22]. According to Javed *et al*. [23] metallic substances are known to mediate free radicals and reactive oxygen compounds, which can cause oxidative damage and/or carcinogenic effects. According to Jomova *et al*. [24] Kurtuas [25] and Stohs & Bagchi [26] redox active metals (Fe, Cu, Cr, Hg, Pb, Cd, and Ni) cause redox cycling, which generates reactive oxygen species (ROS) and damages fish at several levels, including DNA, gills, membrane lipids, and proteins. For certain contaminants, there are unique oxidative stress parameters. For a reliable study, particular oxidative biomarkers must be chosen based on this data. In light of this knowledge, the current study was carried out to evaluate market fish's ability to recognise environmental danger.

2. METHODOLOGY

2.1 Sample Collection and Preservation

With the aid of neighbourhood fishermen, live *Catla catla* (Hamilton) freshwater carp samples were procured from Lower Lake and expertly placed in ventilated polythene carriers stuffed with Lake water. The fish were treated with a 0.05% potassium permanganate solution in the lab to sterilise them. The fishes were then reared in eight cemented tanks and acclimatized using lake water. Mean Dissolved oxygen 6.4 mg/l,pH 7.8,hardness 140.4 mg/l and temperature 28$\pm$2^{o}C) for 14 days. During the acclimatization process, necessary antidotes were applied following prescribed standards to prevent fungal and bacterial contamination. Additionally, the fishes were provided a daily diet of powdered rice bran and groundnut oil cake (3:1 ratio) along with the natural feed available.

The investigation was carried out for a period of 12 weeks (84) days between May- July, 2021.

2.2 Concentration of Heavy Metals Introduced in Experimental Tanks

Catla catla fish species were evaluated based on their tolerance and susceptibility to lake water induced with various heavy metals (Pb, Fe, Zn, Hg, Cr, Cd, Cu, and Ni) at specified concentrations (Table-1) in the laboratory.

The purpose was to observe the aggregation of toxic metals in the Liver and Kidney in *Catla catla* fish, alterations in anti-oxidative enzymes as well as subsequent cellular damage. The experiments involved inducing heavy metals in cemented tanks, each measuring 10 ft X 5 ft X 2.5 ft, placed in a natural environment. A volume of 2000 liters of lake water was added to each tank, and 200 *Catla catla* fish of average length 9.0 cm and weight 80 grams were introduced into each tank for observation. Any deceased fish were promptly removed from the tanks every 48 hours.

In experimental tanks the concentration of dosage (Table-1) of each heavy metal introduced was decided on the basis of range values of that particular heavy metal in the Lake water.

2.3 Superoxide Dismutase (SOD) Activity

2.3.1 Collection of tissues (Liver and Kidney) for the assay

Excised liver and kidney were homogenised in a cold, pH 7.4, 0.25 M sucrose buffer. Following that, the resulting solution was centrifuged for 15 minutes at 4°C using 5000 rpm. To measure the antioxidant enzyme parameter (SOD), the tissue solution was analysed.

Following the epinephrine approach as reported by Sun *et al*. [27] the enzyme activity of Superoxide Dismutase (SOD) in *Catla catla* was assessed spectrophotometrically at an emission wavelength of 480 nm.

2.4 Determination of SOD Activity

By evaluating the proportion of autocatalytic adrenochrome production inhibition at 480 nm in an enzyme medium contains 1 mM adrenaline and 50 mM glycine (pH 10.2), the level of SOD activity was identified. The experiment was conducted for 3 minutes at an even temperature of 30 °C. In superoxide dismutase units per gram of proteins, the enzymatic activity is measured. According to Sun *et al*. [27] a single unit is the quantity of enzymes that suppresses a given proportion of adrenochrome production by 50%. A total of twelve weeks (84 days) of SOD were studied.

2.5 Biomarker Enzyme Analysis

By following normal procedures, the enzymatic biomarkers were analysed. Ellman's [28] spectrometer was used to evaluate the concentration of superoxide dismutase (SOD), which was tested using the Kono *et al*. [29] technique.

One unit of enzymes is equal to 50% inhibition.

$$\text{Inhibition (\%)} = \text{Blank} - \text{Sample} / \text{Blank} \times 100$$

2.6 Statistical Analysis

To acquire the concordant numbers, every study was run three times. For every statistical analysis, GraphPad Prism (version 8) is used. The data are presented as mean ± SD, with a significance level of $P<0.05$ accepted.

3. RESULTS

3.1 Superoxide Dismutase (SOD) Activity in the Fish Exposed to Different Heavy Metals

Superoxide dismutase activity in Liver and Kidney of *Catla catla* fish were exposed to eight Heavy metals is depicted in Table -1. SOD activity was analysed for a period of twelve weeks (84 days).

3.1.1 Lead

SOD values in Liver ranged from 82.6 UmL-1 to 140.75 UmL-1 in exposure with Lead. The minimum value was observed after 14-days whereas maximum value was recorded after 56 days of exposure.

While SOD values in Kidney ranged from 57.68 UmL-1 to 111.43 UmL-1 in exposure with Lead.

The min imum value was observed after 14-days whereas maximum value was recorded after 56 days of exposure (Fig. 1).

3.1.2 Iron

SOD values in Liver ranged from 78.22 UmL-1 to 141.27 UmL-1 in exposure with iron. The minimum value was observed after 14-days whereas maximum value was recorded after 70 days of exposure. While SOD values in Kidney ranged from 45.48 UmL-1 to 133.23 UmL-1 in exposure of Iron. The minimum value was observed after 14-days whereas maximum value was recorded after 70 days of exposure (Fig. 2).

3.1.3 Zinc

SOD values in Liver ranged from 57.28 UmL-1 to 107.24 UmL-1 in exposure with Zinc. The minimum value was observed after 14 days whereas maximum value was recorded after 70 days of exposure. While SOD values in Kidney ranged from 47.66 UmL-1 to 81.32 UmL-1 in exposure with Zinc.

The minimum value was observed after 14-days whereas maximum value was recorded after 70 days of exposure (Fig. 3).

3.1.4 Mercury

SOD values in Liver ranged from 42.21 UmL-1 to 91.92 UmL-1 in exposure with Mercury. The minimum value was observed after 14-days whereas maximum value was recorded after 84 days of exposure. While SOD values in Kidney is ranged from 59.24 UmL-1 to 96.13 UmL-1 in exposure with Mercury. The minimum value was observed after 14-days whereas maximum value was recorded after 70 days of exposure (Fig. 4).

Table 1. Concentration (µg/l) of heavy metals applied to *Catla catla* fish species in cemented experimental tanks

	Heavy Metals introduced	Concentrations in Lake water(µg/l)	Concentrations in Experimental tank water (µg/l)
Tank-1	Pb	0.301	0.383
Tank-2	Fe	2.036	2.242
Tank-3	Zn	1.236	1.414
Tank-4	Hg	0.021	0.042
Tank-5	Cr	0.501	0.614
Tank-6	Cd	0.421	0.481
Tank-7	Cu	0.009	0.011
Tank-8	Ni	0.064	0.074

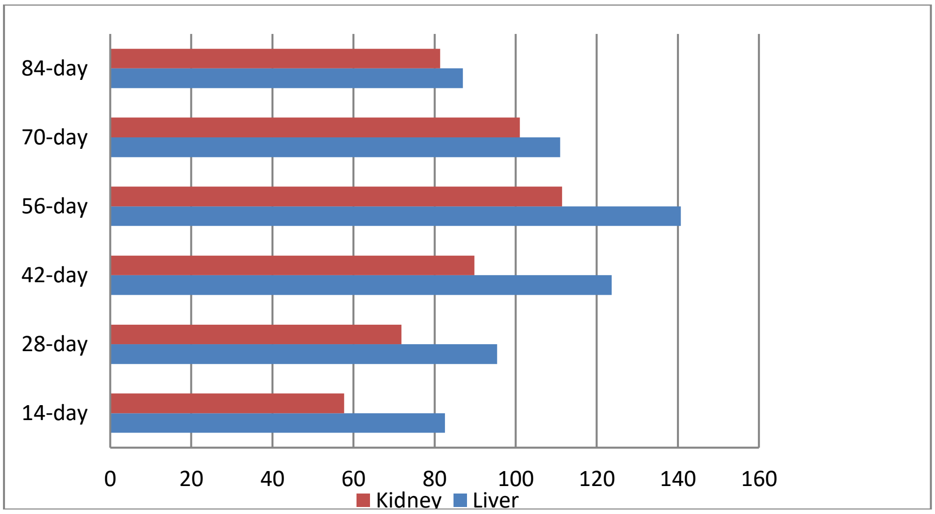

Fig. 1. Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Lead for 84 days

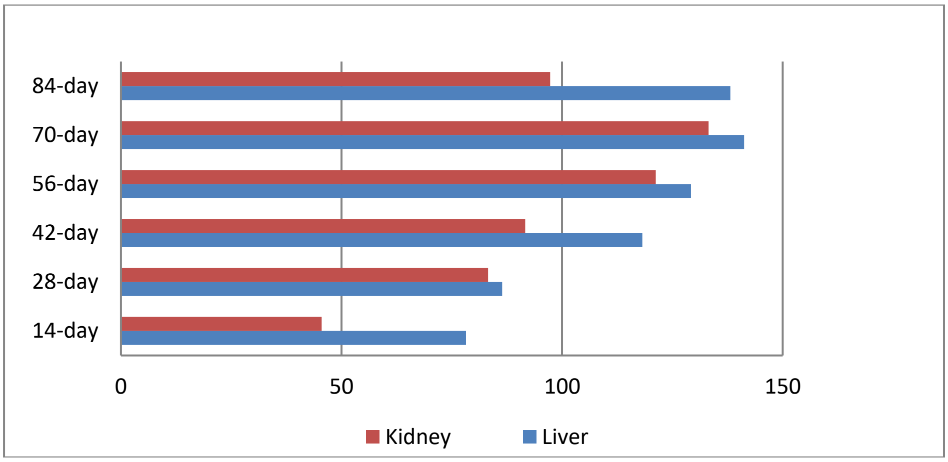

Fig. 2. Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Iron for 84 days

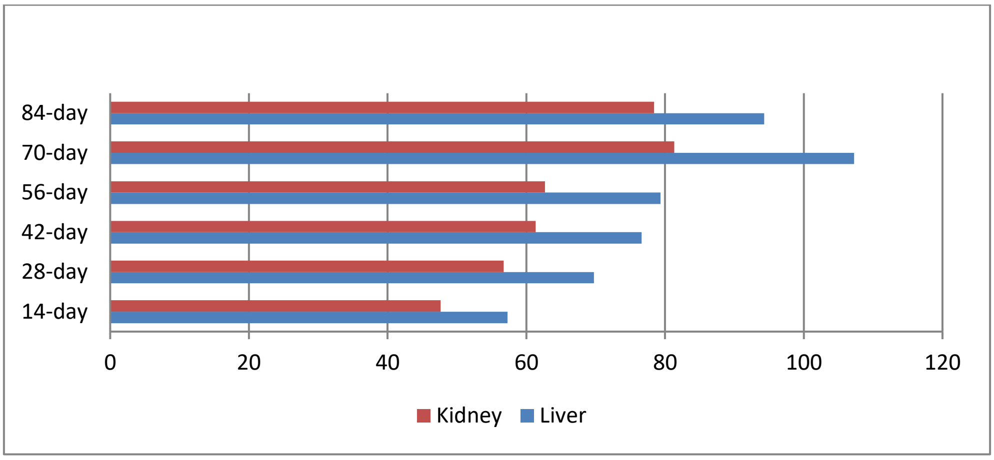

Fig. 3. Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Zinc for 84 days

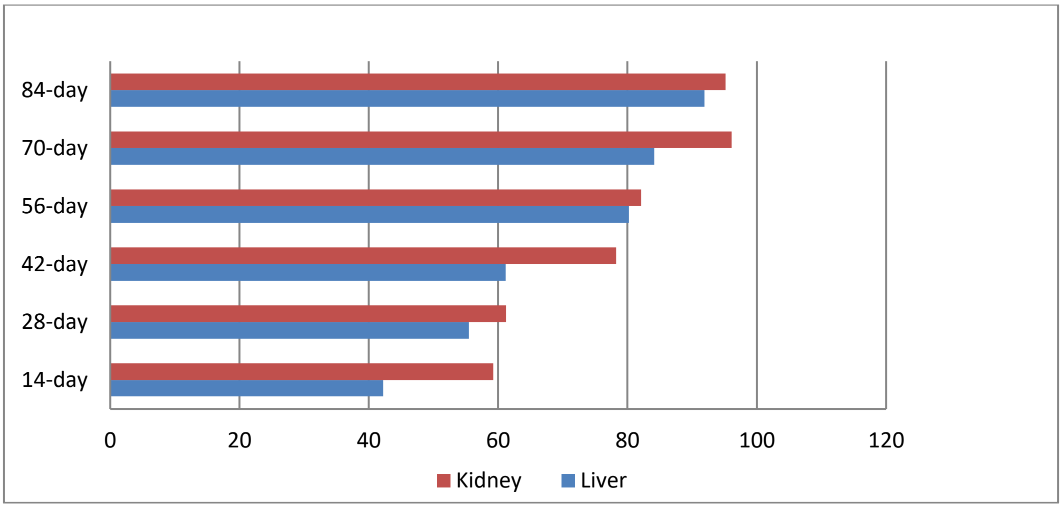

Fig. 4. Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Mercury for 84 days

3.1.5 Chromium

SOD values in Liver ranged from 53.64 UmL-1 to 84.9 UmL-1 in exposure with Chromium. The minimum value was observed after 14-days whereas maximum value was recorded after 70 days of exposure. While SOD values in Kidney ranged from 69.48 UmL-1 to 111.33 UmL -1 in exposure with Chromium. The minimum value was observed after 14-days whereas maximum value was recorded after 70 days of exposure (Fig. 5).

3.1.6 Cadmium

SOD values in Liver ranged from 11.16 UmL-1 to 35.32 UmL-1 in exposure with Cadmium. The minimum value was observed after 14-days whereas a maximum value was recorded after 70 days of exposure. While SOD values in Kidney ranged from 8.2 UmL-1 to 27.67 UmL-1 in exposure with Cadmium. The minimum value was observed after 14-days whereas maximum value was recorded after 70 days of exposure (Fig. 6).

3.1.7 Copper

SOD values in Liver ranged from 20.33 UmL-1 to 93.29 UmL-1 in exposure with Copper.The minimum value was observed after 14-days whereas maximum value was recorded after 84 days of exposure. While SOD values in Kidney ranged from 16.13 UmL-1 to 75.84 UmL-1 in exposure with Copper.

The minimum value was observed after 14-days whereas maximum value was recorded after 84 days of exposure (Fig. 7).

3.1.8 Nickel

SOD values in Liver ranged from 9.5 UmL-1 to 59.2 UmL-1 in exposure with Nickel. The minimum value was observed after 14-days whereas maximum value was recorded after 84 days of exposure. While SOD values in Kidney ranged from 24.74 UmL-1 to 103.48 UmL-1 in exposure with Nickel. The minimum value was observed after 14-days whereas maximum value was recorded after 84 days of exposure (Fig. 8).

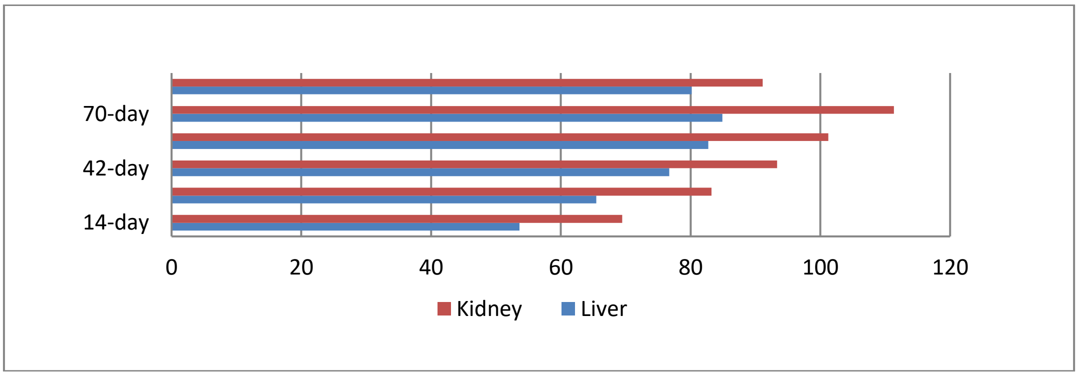

Fig. 5. Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Chromium for 84 days

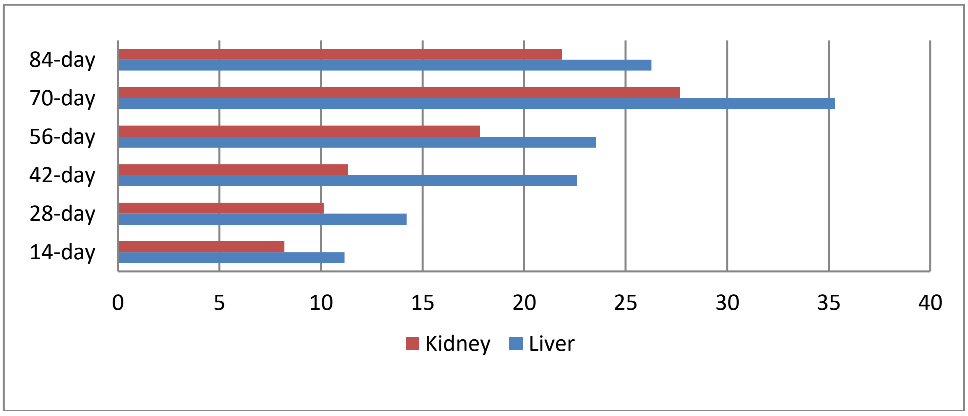

Fig. 6. Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Cadmium for 84 days

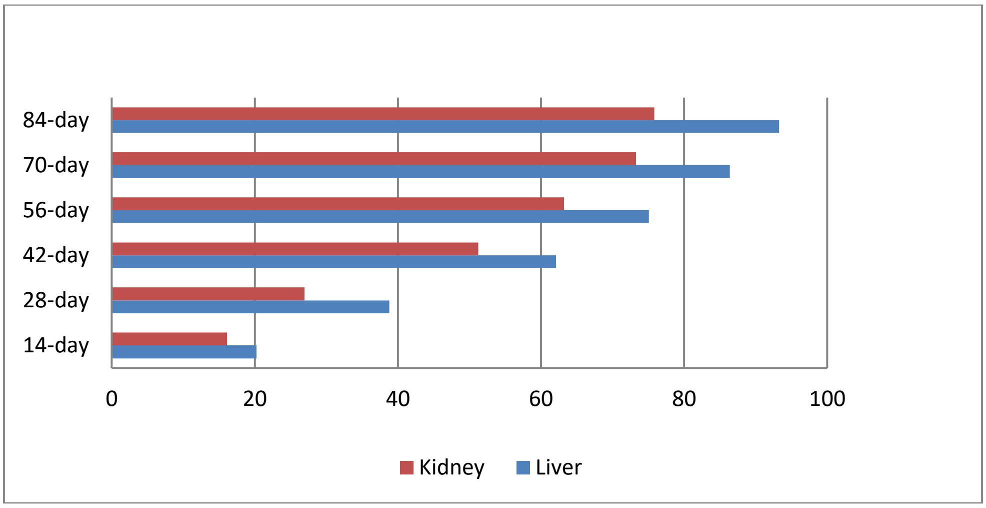

Fig. 7. Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Copper for 84 days

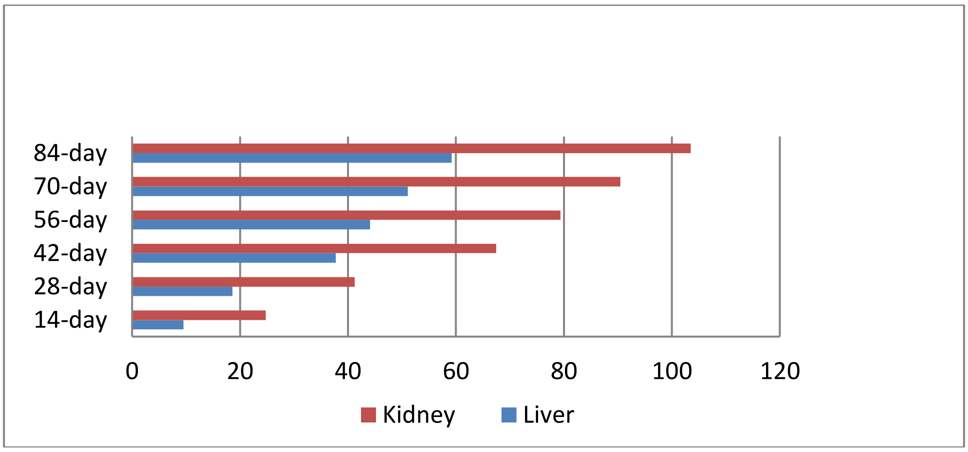

Fig. 8. Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Nickel for 84 days

4. DISCUSSION

The work was carried out to assess the environmental stress in the fresh water fish *Catla catla* at Lower lake Bhopal. This study measures the bioaccumulation of pollutants and its effect on fish health and ecosystem health. In the current study, *Catla catla* fish were exposed to various concentrations of heavy metals to observe changes in antioxidant enzyme (SOD) activities in liver and kidney tissues. The concentration of metals in the fish tissues is depicted in (Table1). During the period of investigation the accumulation of heavy metals in the tissues of the treated fish species varied significantly on exposures of different concentrations of heavy metals in tank waters. Maximum mean sensitivity of *Catla catla* to heavy metal bioaccumulation from different concentrations followed the order: Concentration of metals in Liver fallows as Fe> Pb> Zn> Cu>Hg> Cr> Ni> Cd and concentration in Kidney was Fe> Pb> Cr> Ni> Hg>Zn> Cu> Cd. These heavy metals showed the additive effects on the sensitivity of the *Catla catla* fish species.

In current study the fish exposed to heavy metals resulted in oxidative stress, indicating that the fish uses enzymatic mechanisms to cope with the effects caused by reactive oxygen species (ROS) due to metal accumulation. Findings of existing study are in accordance with the findings of Livingstone [30] who observed similar results that the oxidative stress caused by pesticides in aquatic organisms may lead to ROS production and alterations in antioxidants enzymes. The increased SOD activity in the current study indicates cellular damage in the tissues of metal-treated fish *Catla catla*. The present findings are in accordance with the findings of Sureda *et al*., [31] ; Dorts *et al*., [32] who reported that the cellular antioxidant mechanisms can reduce excessive ROS generation and its harmful consequences.The study observed that the increase in SOD in Kidney and liver in the Metal treated fish indicates a detoxifying mechanism against the toxicity, these findings are in consonance with the findings of Monteiro *et al*., [33] Modesto and Martinez, [34] who stated that the organs of *Brycon cephalus* subjected to Methyl parathion showed a similar effect as well. The current study examined tissue-specific changes in the activity of antioxidant enzymes like SOD being exposed to heavy metals, indicating that various tissues may generate free radicals at varying rates and possess varied antioxidant potentials. The current findings are in conformity with the findings of Monteiro *et al.* [35] stated that the differing levels of free radical formation and antioxidant potentials of various tissues are shown by the tissue-specific changes in the activity of antioxidant enzymes like SOD and catalase that were found following Methyl Parathion exposure. In *Catla catla*, contact with metals has a significant potential to cause oxidative damage. The study are also in consonance with the observations of Abhijith *et al*.,[36] who reported that the fluctuation in SOD activity during the exposure in the current investigation may have been caused by various organ response patterns, such as sensitiveness, gathering, and elimination mechanisms.

The findings of this study demonstrate that *Catla catla* enzyme profiles are significantly altered as a result of exposure to certain heavy metals present in tank water. The ecology and the ability of fish to survive are both threatened by the high concentrations of toxic metals in the aquatic ecosystem. The results of this research on *Catla catla* can be used to compare the reactions of several biomarkers in species that live in contaminated settings. When determining the toxicity of metals in aquatic habitats, these measures might be employed as biomarkers.

The current study reflected the conformity with the findings of Nagaranian *et al*. [37] who observed that when heavy metals or contaminants are present, animals often experience oxidative stress. They need oxidative defence systems like enzymes and chelation to maintain cellular ionic equilibrium, which is disrupted. The current findings corroborate the findings of (McCord and Fridovich, 1969) Winston and Di Giulio, 1991 who reported that in any stressed circumstance, SOD is the main enzyme that animals use to combat oxidative stress. Fish subjected to metal had higher levels of SOD in their liver and kidney, which is a sign of a detoxifying defence against poisoning. The present study also supported by Hegazi *et al*., [38] who reported that the liver and kidney damages brought on by the ROS produced as a consequence of contact with toxic metals may have led to the leaking of these proteins in the plasma. In general, increased antioxidant activity denotes individuals' adaptive mechanisms to combat the oxidative consequences of produced ROS. The current findings are in line with the findings of Gravato *et al*., 2006) who evaluated that the antioxidant enzymes are often used by stressed organisms to adjust to external stress, and their activity depend on the dosage, species, and being subjected.

The investigation's findings imply that the fish uses enzymatic defences to withstand the impacts of ROS produced by heavy metal buildup.The changes in enzymatic parameters can serve as effective biomarkers for monitoring heavy metal pollution in the aquatic environment**.**

5. CONCLUSION

There is a growing concern that the elements through the natural cycling process are being disturbed by anthropogenic activities, especially the growth of industrial, domestic and urban discharge of its effluents. From the present study, we conclude that SOD can be used as the biomarker for Heavy metal contamination. Ultimately, these studies must focus on measuring levels of pollution that may induce irreversible ecological changes to aquatic ecosystems. Till now the levels of toxicity were moderate, and it was progressing toward the danger. Efforts can be made to maintain and control the activities that release pollutants unnaturally into the environment from both public and government so that the clean and clear environment can be maintained.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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