Enzymatic Modifications (SOD) As Biomarker Of Heavy Metal Toxicity In Fresh Water Fish, *Catla catla*

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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. 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 *Catlacatla* fish.

Keywords: Heavy metal toxicity;*Catlacatla;*Biomarkers;Antioxidant enzyme(SOD);Oxidative stress; ROS

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1.Introduction

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Concerns over environmental issues, particularly water contamination, have grown around the world throughout the past three decades. According to Afshan *et al*. (2014), Garg *et al*. (2009), and Nagarani *et al*. (2020), ~~and other authors~~, 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*. (2011), 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*., 2010; Arkoosh*et al*., 2010). 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, 2017). 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*. (2013), 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*. (2010), 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*., 2021). Through the formation of metallic complexes, toxic metals impede the activities of structural amino acids, digestive enzymes, and DNA (Jaishankar *et al*., 2014). Furthermore, it also causes genomic abnormalities, structural or physiological modifications, and immunological system dysfunction (Coen *et al*., 2012). 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 (Tao *et al*., 2001).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 (AL-Yousuf *et al*., 2000).According to Javed *et al*. (2015), 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*. (2010), Kurtuas (2015), and Stohs & Bagchi (1995), 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

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Sample collection and preservation

The wholesome, fresh *Catlacatla* fish were purchased at 6 a.m. from the fish market, Lower Lake Bhopal,M.P,India. The investigation was carried out between January 2020 and December 2021. The study employed physical evaluation to choose the samples, which included fully developed fish (fingerlings were not included), fresh and devoid of rotting odour, red-coloured gills, soft tissue without mucus (which indicates the existence of bacteria or pathogens), and chemical-odour-free. The moment samples were gathered, they were kept in ice-cold (4 °C) storage. Two *Catlacatla*specimens from each tank were utilised for each experiment.For metal evaluation, fishes were carefully cleaned in distilled water that had been sterilised and then oven-dried. Utilising an atomic absorption spectrometer, the metallic levels were measured in accordance with the accepted double-acid digestion procedures. Utilising certified preparations (Merck, UK) processed with HNO 3 to a similar pH as the specimens, standards were created. New samples were kept for upcoming biochemical research at −80 °C.

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~~A.~~ Superoxide Dismutase ~~Activity~~ (SOD) Activity

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*. (2006), the enzyme activity of Superoxide Dismutase (SOD) in *Catlacatla* was assessed spectrophotometrically at an emission wavelength of 480 nm.

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.* (2006) ,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.

Biomarker enzyme analysis

By following normal procedures, the enzymatic biomarkers were analysed. Ellman's (1961) spectrometer was used to evaluate the concentration of superoxide dismutase (SOD), which was tested using the Kono *et al.* (2000) technique.,

One unit of enzymes is equal to 50% inhibition.

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

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

~~The work was carried out to assess the environmental stress in the fresh water fish*Catlacatla* at local market. This study also measures the bioaccumulation of pollutants and its effect on fish health and ecosystem health.~~

A. Superoxide Dismutase ~~Activity~~ (SOD) Activity in the fish exposed to different Heavy Metals

Superoxide dismutase activity in Liver and Kidney of *Catlacatla* fish were exposed to eight Heavy metals is depicted in Table1 and Figure: 1. SOD activity~~A~~ was analysed for a period of twelve weeks (84 days)

1. Lead

SOD values in Liver ~~of Lead 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 ~~of~~ Lead ~~in Kidney ranged from 57.68 UmL-1 to 111.43 UmL-1.~~

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

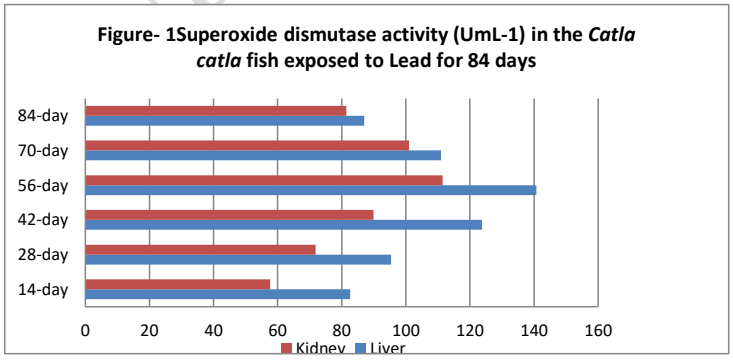

Figure- 1Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Lead for 84 days

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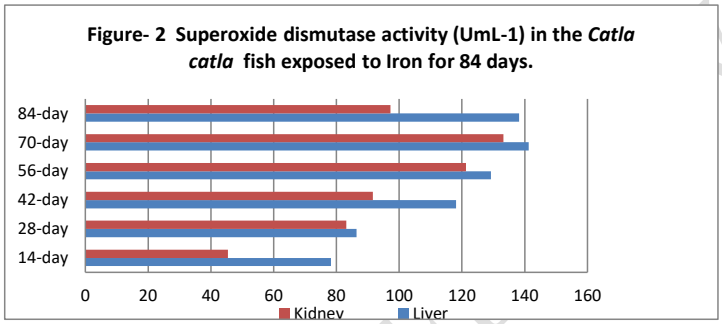
2. Iron

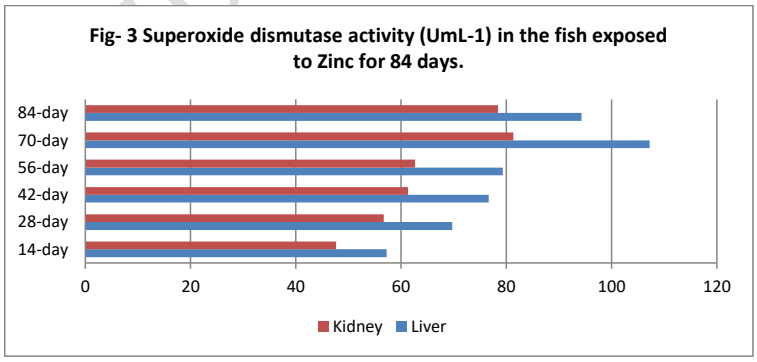
SOD values of **Iron** in Liver ranged from 78.22 UmL-1 to 141.27 UmL-1. The minimum value was observed after 14-days whereas maximum value was recorded after 70 days of exposure. While SOD values of **Iron** in Kidney ranged from 45.48 UmL-1 to 133.23 UmL-1. The minimum value was observed after 14-days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



3. Zinc

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

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



4. Mercury

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

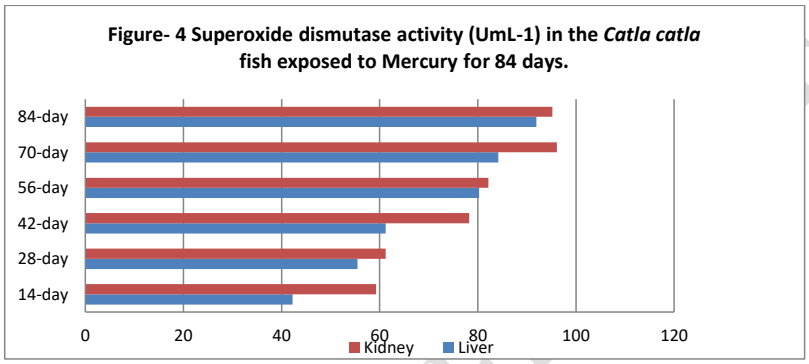


Figure- 4 Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Mercury for 84 days.

5. Chromium

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

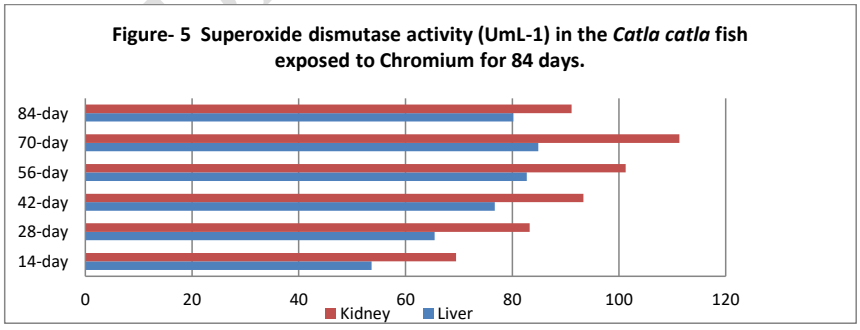


Figure- 5 Superoxide dismutase activity (UmL-1) in the *Catla catla* fish exposed to Chromium for 84 days.

6. Cadmium

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

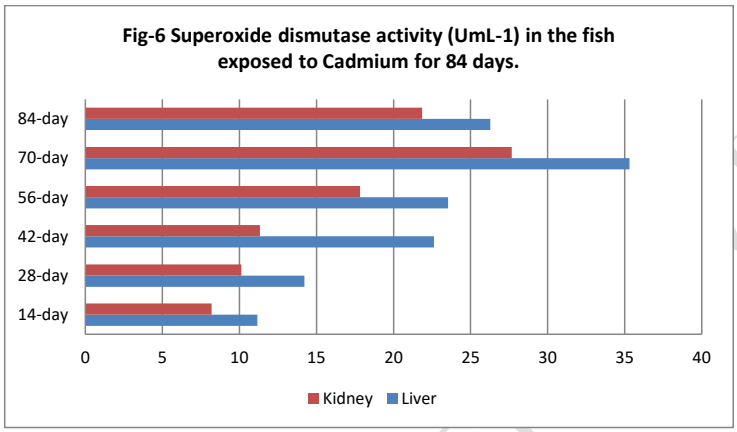
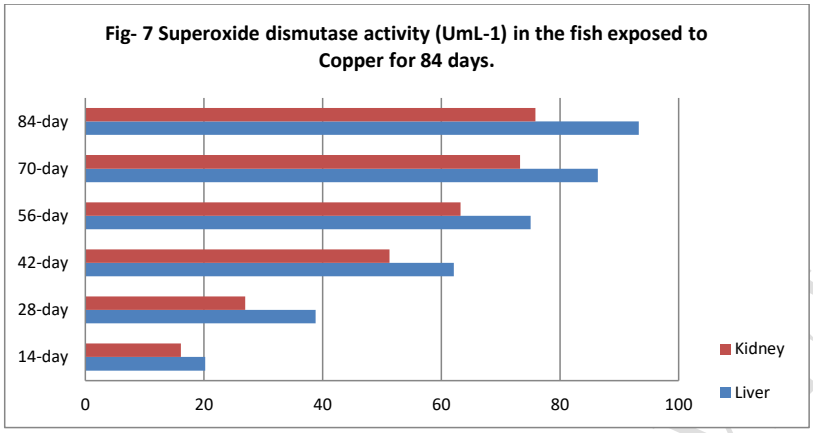


Fig-6 Superoxide dismutase activity (UmL-1) in the fish exposed to Cadmium for 84 days.

7. Copper

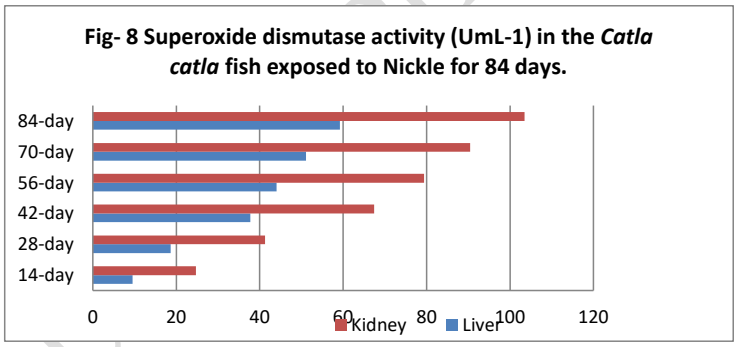
SOD values of **Copper** in Liver ranged from 20.33 UmL-1 to 93.29 UmL-1. The minimum value was observed after 14-days whereas maximum value was recorded after 84 days of exposure. While SOD values of **Copper** in Kidney ranged from 16.13 UmL-1 to 75.84 UmL-1.

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



8. Nickel

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



~~Enzymes play an important role during the metal toxicity in eliminating and converting the free radical into stable molecule and thus prevent cellular damage~~.The concentration of metals in the fish tissues is depicted in Figure. During the period of investigation the accumulation of heavy metals in the tissues of the treated fish species varied significantly

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on exposures of different concentrations of heavy metals in tank waters. Maximum mean sensitivity of *Catlacatla*to heavy metal bioaccumulation from different concentrations followed the order: Hg>Pb>Cd>Cr >Zn> Ni>Cu.These heavy metals showed the additive effects on the sensitivity of the *Catlacatla*fish species.

In the current study, *Catlacatla* fish were exposed to various concentrations of heavy metals to observe changes in antioxidant enzyme (SOD) activities in liver and kidney tissues. The exposure 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. The increased SOD activity indicates cellular damage in the tissues of metal-treated *Catlacatla*.The study also found 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. In *Catlacatla*, contact with metals has a significant potential to cause oxidative damage.

The findings of this study demonstrate that *Catlacatla*'s 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 *Catlacatla* 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.

4. Discussion

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. (Nagaranian *et al*. (2009).In any stressed circumstance, SOD is the main enzyme that animals use to combat oxidative stress (McCord and Fridovich, 1969; Winston and Di Giulio, 1991). They noticed that 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 organs of *Brycon cephalus* subjected to MP showed a similar effect as well (Monteiro *et al*., 2009; Modesto and Martinez, 2010). Elevated enzyme activity of SOD in male rats following atrazine treatment suggests a compensatory response to elevated ROS production (Singh *et al*., 2011). According to the research, the liver and kidney damages brought on by the ROS produced

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as a consequence of contact with toxic metals may have led to the leaking of these proteins in the plasma in the current experiment. In general, increased antioxidant activity denotes individuals' adaptive mechanisms to combat the oxidative consequences of produced ROS (Hegazi *et al*., 2010). Cellular antioxidant mechanisms can reduce excessive ROS generation and its harmful consequences (Sureda *et al*., 2004; Dorts*et al*., 2012).Antioxidant enzymes are often used by stressed organisms to adjust to external stress, and their activity depend on the dosage, species, and being subjected (Gravato *et al*., 2006). The fluctuation in SOD activity during the ~~over the duration of~~ exposure in the current investigation may have been caused by various organ response patterns, such as sensitiveness, gathering, and elimination mechanisms.

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~~s~~ 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.

Consent for publication Not applicable.

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