RESEARCH ARTICLE

Ecotoxicological Effects of Lead Exposed *Cyprinus carpio* and HSP70-Induced Antioxidants against ROS

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ABSTRACT

Lead (Pb) is one of the leading heavy metal pollutions in freshwater sources. This study summarises lead accumulation in freshwater fish *Cyprus carpio* and its toxicological effects. Pb is known to induce neuro, nephro, and hepatotoxicity in *C. carpio* and, finally, humans. The effect of Pb leads to the generation of reactive oxygen species (ROS) and antioxidants in the tissues of *C. carpio*. Antioxidant assays revealed the extent of free radical scavenging activity of the infected tissues. LC_{50} at a time interval of 24 hours showed a concentration of 7.919 ppm. ROS analysis revealed that the highest concentration of Pb toxicity was observed in the kidneys, liver, and brain of *C. carpio*. Not only free radicals but also Pb toxicity have been known to activate heat shock proteins as a result of oxidative stress management. This study has raised alarming responses to freshwater aquaculture to initiate control measures against the toxicity and prevent its further entry into the food chain.

Keywords: Antioxidants, *Cyprinus carpio*, Lead, Nephrotoxicity, Neurotoxicity, Oxidative stress, Toxicity.

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INTRODUCTION

The primary sources of pollutants include industrial waste discharge, agriculture, mining, home waste disposal, and fuel combustion.[1] By implementing current fish culture techniques, a spectacular breakthrough has been accomplished in the field of aquaculture, resulting in a significant increase in the output of culture fishes. In the years 2002–2003, fish is a nutritious delicacy that is eaten as a delicacy across the country. However, adding contaminants to water influences the aquatic ecology and harms fish health. As a result, fish productivity is often hampered, and fishermen confront significant challenges, including economic losses. Bioaccumulation in fishes, oysters, mussels, and other aquatic ecosystem components has been observed all over the world. Animals' sensitivity and survival potential to certain harmful compounds, such as heavy metals, may be measured using lethal concentration of 50% (LC_{50}) studies. While a higher concentration of heavy metals is required to induce 50% death in animals, higher LC_{50} values are generally less hazardous. Recent studies have recently implicated free radicals and reactive oxygen species (ROS). Heat shock proteins (HSPs) are also involved in oxidative stress defense.[2] Heavy metals like lead and mercury generated reactive oxygen species, which in turn caused neurotoxicity, hepatotoxicity, and nephrotoxicity in fish target tissues. Thus, the present study aims to study the acute toxicity and LC_{50} value of lead on freshwater fish *Cyprinus carpio* (Common carp) from a freshwater pond in Bengaluru, Karnataka.

MATERIALS AND METHODS

Sample Collection

An 18 2.0 g *C. carpio* was collected from a pond in Bengaluru, Karnataka. Morphological characteristics served as the basis for the selection criterion for selecting healthy fish. Before the start of the real experiment, the fish were acclimated at 32°C with a natural photoperiod and fed with commercial feed once a day. The fish was initially measured for length and body weight. According to established protocols, the water quality was initially checked for total dissolved solids and other physicochemical parameters.[3]

Sample Preparation and Water Analysis

The liver, brain, and kidney tissue samples of *C. carpio* were removed, and by using an acetone extraction/microwave digestion procedure, protein powders that had been partially defatted were produced. To ensure the absence of lead (Pb), the physicochemical characteristics of the freshwater used to grow *C. carpio* in a lab setting were examined.

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Toxicity Studies

Finney probit analysis was used to investigate the acute toxic effects of lead on *C. carpio*. The percentage of mortality was computed, and the results were converted to a Probit scale and analyzed accordingly.[4]

Detection of Reactive Oxygen Species

The effect of ROS as a result of Pb exposure on the tissues of *C. carpio* was studied. The tissue samples (Liver, Brain, and Kidney) were rinsed with phosphate-buffered saline (PBS) and digested with 0.5% trypsin and 0.1% collagenase for about 40 minutes. This solution was subjected to centrifugation at 600 rpm for 5 minutes and re-suspended in Hank's buffer. Roughly 1×10^6 cells were washed with PBS and re-suspended in Hank's buffer. To each sample, 40 µL of dichlorofluorescein diacetate (DCFH-DA: 2.5 mmol/L) was added and incubated at 37°C for 30 minutes in the dark. An inverted fluorescent microscope (Olympus CKX 41) was used to detect the relative quantities of H_2O_2 generated after the cells had been thoroughly washed to remove surface fluorescence. A 100 W xenon lamp and fluorescein isothiocyanate filter set were used to calibrate the background to zero, and image analysis software was then used to measure the output.

In-vitro Antioxidant Assay

Catalase activity

The catalase (CAT) activity was determined using the standard protocol of.[5] To 1-mL of the tissue homogenate of *C. carpio*, 1-mL of phosphate buffer (pH 7.0), and 0.5 mL of hydrogen peroxide (H_2O_2) were added. Absorbance was measured at 530 nm and the amount of protein was expressed as µ/mg.

Superoxide dismutase assay

The superoxide dismutase (SOD) assay was performed based on.[6] In a total volume of 3 mL, the assay combination included 1.2 mL sodium pyrophosphate solution, 0.1 mL phenazine methosulphate, 0.3 mL nitro blue tetrazolium NBT, 0.2 mL NADH, 1-mL of tissue extract of *C. carpio*, and 0.4 mL of distilled water. NADH was added to initiate the reaction. This solution was incubated at 30°C for 150 seconds. Further, the reaction was ceased by the addition of 1-mL of glacial acetic acid. To the reaction mixture, 4 mL of n-butanol was added and shaken vigorously. The supernatant from the centrifugation of this solution at 13,000 rpm for 15 minutes was used to measure the chromogen intensity at 560 nm.

Glutathione reductase assay

David and Richard (1983)[7] method was used to estimate glutathione reductase activity. To 1-mL of potassium buffer (0.12 M, pH 7.2, 0.1 mL of sodium azide), 0.1 mL of EDTA, and 0.1 mL of oxidized glutathione were added to 0.1 mL of enzyme extract. Using distilled water, the total assay volume was attuned to 2 mL. The liquid was held at room temperature for 3 minutes to initiate the reaction and 0.1 mL of NADPH was added. At 15 seconds intervals, the absorbance was measured at 340 nm for 2 minutes. The enzyme activity was measured in moles of NADPH oxidized per minute per mg of the enzyme.

RESULTS

Physicochemical Analysis of Freshwater

Dissolved salts and hazardous metals were examined in the freshwater used to raise *C. carpio*. The test revealed that the dissolved oxygen level was 6.2 ± 0.4 mg/l with a neutral pH of 7.3 ± 0.01. The total water hardness was found to be 345 ± 99 mg/l. Meanwhile, the free CO_2 was estimated to be 2.1 ± 0.12 mg/L. There were no traces of mercury and cadmium present in the tested water. However, traces of calcium (81 ± 88 mg/L) and magnesium (34 ± 0.0 mg/L) were noticed. Also, high amounts of sulfates and chlorides were detected in the water sample (Table 1). This test proved that *C. carpio* was not exposed to lead before the start of the experiment.

Estimation of Lethal Concentration

The lethal concentrations of lead exposure were checked for a time duration ranging from 24 to 96 hours. A significant decrease in the LC values for almost all the LC ranging from LC_5, LC_{10}, LC_{16}, LC_{50}, LC_{84}, LC_{90}, to LC_{90} was examined. In the case of LC_{50} concentration, the values gradually decreased from 7.919, 7.064, 6.981, 5.417 ppm on 24th, 48th, 72nd, and 96th hour of exposure, respectively (Figure 1).

Table 1: Physicochemical characteristics of the water used for growing *C. carpio*

Parameters	*Values*
Dissolved oxygen	6.2 ± 0.4 mg/l
pH	7.3 ± 0.01 m
Temperature	28 ± 2°C
Total hardness	345 ± 99 mg/l
Free CO_2	2.1 ± 0.12 mg/l
Ca	81 ± 88 mg/l
Mg	34 ± 0.0 mg/l
Hg	Nil
Sulfates	112 ± 0.9 mg/l
Chlorides	234 ± 22 mg/l
Pb	Nil
Specific conductance	2340 (Micro siemens/cm) at 2°C

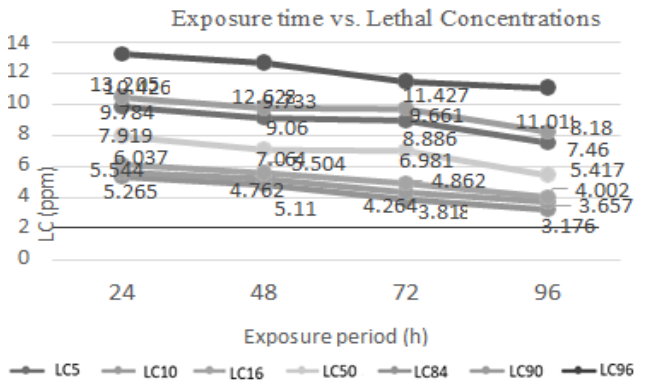

Figure 1: Graph showing the effect of toxicity of lead estimated as exposure time versus lethal concentrations

Probit Regression Analysis

Probit regression analysis was performed to ascertain the fish's lethal concentration of lead exposure. The value of the LC decreased as the exposure period increased. Therefore, it can be concluded that the longer the exposure time, the more the dependent variable slope function (S) increases (Table 2).

ROS Analysis

In ROS analysis, the intensity of fluorescence of dye present in the cells is directly proportional to the free radicals released. In Figure 2a, a higher number of free radicals were generated in the lead-treated liver of the fish when compared to the control. Meanwhile, a similar intensity was noticed in the brain cells of the fish treated with lead compared to the control fish (Figure 2b). Also, in the kidneys, high levels of free radicals were noted (Figure 2c).

Table 2: Probit regression analysis

Exposure period (h)	*Correlation coefficient*	*Slope function (S)*	*Confidence level*		*Chi- square test*
			Upper	*Lower*	
24	0.975	1.349	8.005	7.627	4.486
48	0.961	1.379	7.453	6.881	6.498
72	0.959	1.206	6.296	6.107	6.214
96	0.937	1.47	5.773	5.266	5.969

$p < 0.05$ level of significance.

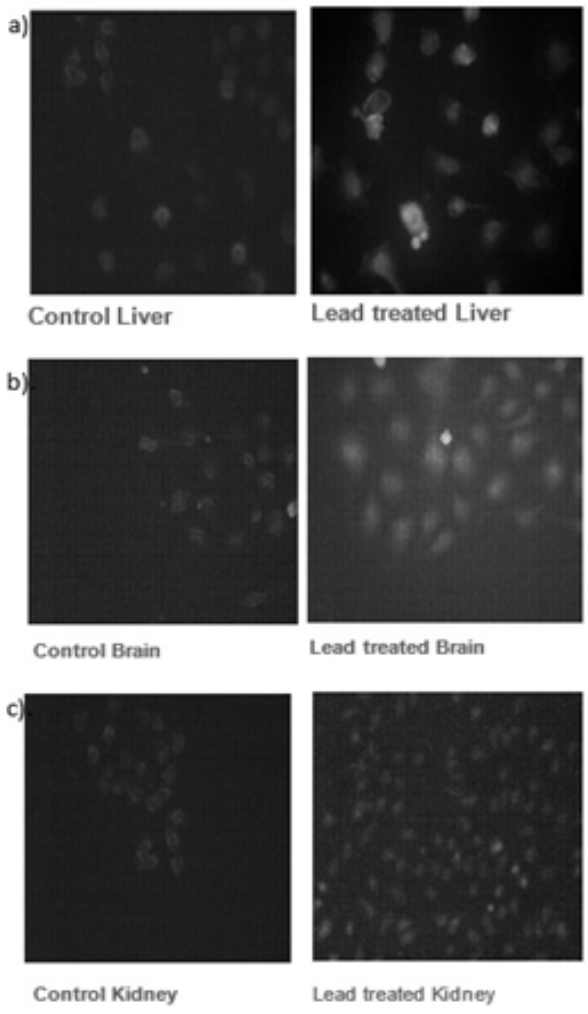

Figure 2: ROS analysis

Analysis of Catalase Activity

Catalase (CAT) activity increases with an increase in the days of exposure to lead in liver, brain, and kidney. The liver shows a prominent increase in CAT activity from 09.52 ± 0.89 µ/mg protein on the 0^{th} day to 12.84 ± 6.02 µ/mg protein on the 28^{th} day (Table 3). In contrast, the CAT activity in the brain increased to 7.39 ± 3.44 µ/mg protein on the 28^{th} day, significantly less than the activity in the kidneys (9.63 ± 4.84 µ/mg protein).

Estimation of Superoxide dismutase in Activity

The highest SOD activity of 2.74 ± 0.21 µ/mg protein was observed in the liver of *C. carpio* on the 28^{th} day of testing. However, comparatively less activity was noticed in the brain and kidneys of the fish (Table 4).

Assessment of Glutathione Reductase activity

It was noticed that there was an increase in glutathione reductase activity with the increase in lead exposure in liver brain and kidney. The kidney shows a significant increase from 0.97 ± 0.89 µ/mg protein on 0^{th} day to 1.23 ± 0.77 µ/mg protein on 28^{th} day. However, GR activity was comparatively lower in the case of the liver and brain of *C. carpio.*

DISCUSSION

Lead is the most hazardous heavy metal and is soluble in water depending on pH, salinity, and hardness. The solubility of lead is highest in soft and acidic water. Lead accumulation mainly occurs in the liver, spleen, kidney, and gills.

This study investigated the oxidative stress and genotoxic effects of lead in the kidney, liver, and brain of a common freshwater fish, *C. carpio*. Alterations in levels of ROS, SOD, CAT, and GR were used as indicators of lead toxicity. The Table 3 showing Estimation of CAT activity in liver, brain, and kidney of C. carpio treated with lead. The effect of DNA damage that occurred was also measured due to lead exposure. This study examined the toxicological effects of exposure to sub-lethal doses. Sub-lethal concentrations (1/2, 1/5, 1/10, and 1/15) of the 96 hours LC_{50} were selected and the fishes were exposed to each concentration throughout 7, 14, 21, and 28 days. Metal exposure produces a superoxide anion radical, which is decomposed by SOD into hydrogen peroxide. Table 4 showing the SOD activity in the liver, brain, and kidney of *C. carpio* treated with lead. Increased levels in SOD and CAT activity, as well as ROS content, were seen in all three tissues after 28 days of exposure, but only at the maximum concentration of exposure. Lead toxicity causes oxidative stress in fish, resulting in synaptic damage and neurotransmitter dysfunction. Many studies have found that Pb exposure enhanced or lowered SOD activity in fish.[8] Due to the

Table 3: Estimation of CAT activity in liver, brain, and kidney of *C. carpio* treated with lead

Catalase activity (μ/mg protein)						
Duration/Time interval (Days)	*Liver*		*Brain*		*Kidney*	
	Control	*Test*	*Control*	*Test*	*Control*	*Test*
0	09.52 ± 0.89	09.52 ± 0.89	06.18 ± 0.52	06.18 ± 0.52	08.42 ± 0.81	08.42 ± 0.81
7	09.52 ± 0.89	10.95 ± 4.27	06.18 ± 0.52	06.34 ± 4.43	08.42 ± 0.81	08.69 ± 6.45
14	09.52 ± 0.89	11.33 ± 3.21	06.18 ± 0.52	06.84 ± 3.58	08.42 ± 0.81	08.84 ± 4.84
21	09.52 ± 0.89	12.14 ± 3.47	06.18 ± 0.52	06.99 ± 2.91	08.42 ± 0.81	09.02 ± 4.57
28	09.52 ± 0.89	12.84 ± 6.02	06.18 ± 0.52	07.39 ± 3.44	08.42 ± 0.81	09.63 ± 4.84

Values are expressed as Mean ± SD for n = 3.

Table 4: SOD activity in the liver, brain, and kidney of *C. carpio* treated with lead

Superoxide dismutase (μ/mg Protein)						
Duration/Time Interval (Days)	*Liver*		*Brain*		*Kidney*	
	Control	*Test*	*Control*	*Test*	*Control*	*Test*
0	1.41 ± 0.14	1.41 ± 0.14	0.99 ± 0.35	0.99 ± 0.35	1.09 ± 0.27	1.09 ± 0.27
7	1.41 ± 0.14	1.72 ± 0.21	0.99 ± 0.35	1.06 ± 0.26	1.09 ± 0.27	1.19 ± 0.31
14	1.41 ± 0.14	2.29 ± 0.28	0.99 ± 0.35	1.13 ± 0.28	1.09 ± 0.27	1.23 ± 0.49
21	1.41 ± 0.14	2.48 ± 0.14	0.99 ± 0.35	1.39 ± 0.36	1.09 ± 0.27	1.27 ± 0.28
28	1.41 ± 0.14	2.74 ± 0.21	0.99 ± 0.35	1.54 ± 0.51	1.09 ± 0.27	1.46 ± 0.42

Values are expressed as Mean ± SD for n = 3

Table 5: GR activity in liver, brain, and kidney of *C. carpio* treated with lead.

Glutathione Reductase (μ/mg Protein)						
Duration/Time Interval (Days)	*Liver*		*Brain*		*Kidney*	
	Control	*Test*	*Control*	*Test*	*Control*	*Test*
0	1.31 ± 0.54	1.31 ± 0.54	0.72 ± 0.16	0.72 ± 0.16	0.97 ± 0.89	0.97 ± 0.89
7	1.31 ± 0.54	1.37 ± 0.52	0.72 ± 0.16	0.74 ± 0.26	0.97 ± 0.89	1.05 ± 0.73
14	1.31 ± 0.54	1.46 ± 0.54	0.72 ± 0.16	0.78 ± 0.35	0.97 ± 0.89	1.06 ± 0.89
21	1.31 ± 0.54	1.48 ± 0.63	0.72 ± 0.16	0.83 ± 0.15	0.97 ± 0.89	1.14 ± 0.89
28	1.31 ± 0.54	1.55 ± 0.59	0.72 ± 0.16	0.92 ± 0.18	0.97 ± 0.89	1.23 ± 0.77

Values are expressed as Mean ± SD for n = 3.

defensive mechanisms against ROS generation, SOD activity in fish subjected to Pb is normally elevated.[8] Pb exposure, on the other hand, might reduce SOD function by reducing antioxidants or lowering hydrogen peroxide. Redox-inactive metals like Pb, Hg, and Cd lower antioxidant levels in the cell substantially, whereas redox-active metals like Cr, Fe, and Cu undergo redox cycling. Hydrogen peroxide is decomposed into water and oxygen by CAT and glutathione peroxidase (GPx). Reduced glutathione (GSH) is converted to GSH disulfide (GSSG) during the process of GPx decomposition. In metabolic detoxification, GSH aids in the elimination of ROS and cofactors. Many researchers have reported GSH levels in fish exposed to Pb to rise or decrease significantly[8] Table 5 showing the GR activity in liver, brain, and kidney of *C. carpio* treated with lead.

GSH levels in fish exposed to Pb can be decreased due to Pb binding to –SH groups, which reduces GSH's antioxidant activity; yet, it can be raised due to oxidative stress produced by Pb exposure, which activates antioxidants. By catalyzing the conjugation of xenobiotics, GST helps remove them from the biological system.[8] Pb binds thiol (-SH) groups, which function as antioxidants in cells and are found in a variety of proteins and enzymes, including GSH. Lead exposure causes oxidative stress and the generation of ROS in fish, and antioxidant responses, including SOD, CAT, GSH, GST, and TBARS are important markers of oxidative stress in Pb-exposed fish. During the experiment, no noticeable changes in CAT, SOD, or GR were identified in any of the treatments. After persistent exposure to mesotrione, comet tests demonstrated that the greatest concentration of mesotrione caused DNA damage in many organs in common carp, particularly the liver. These findings show that the oxidant-antioxidant and comet assays might be used to determine the toxicity of water contaminants in monitoring systems. Lead build-up in the brain is higher than in the kidney and liver, according to EDAX and heavy metal analyses. The African catfish *Clarias gariepinus* was

shown to have histological gill and liver tissue deformation after being exposed to lead.

CONCLUSION

In conclusion, it is apparent from the present study that Pb toxicity has a deleterious effect on *C. carpio*, affecting the food chain when consumed by humans. It causes neurotoxicity, nephrotoxicity, and hepatotoxicity not only in fish but also in humans. It induces oxidative stress and increases HSP levels in *C. carpio*. These data also suggest that Pb may stimulate ROS, and that HSP70 produces antioxidants in *C. carpio* as a countermeasure. As a result, mandated testing of freshwater fish exposed to heavy metal contamination will aid in stopping the metal's passage down the food chain and protecting the aquatic ecosystem.

REFERENCES

1. Saxena R, Garg P. Vitamin E provides protection against in vitro oxidative stress due to pesticide (Chlorpyrifos and Endosulfan) in goat RBC. Bull Biosci. 2010;1:1-6.
2. Song G, Yuan S, Wen X, Xie Z, Lou L, Hu B, Cai Q, Xu B. Transcriptome analysis of Cd-treated switchgrass root revealed novel transcripts and the importance of HSF/HSP network in switchgrass Cd tolerance. Plant cell reports. 2018 Nov;37:1485-1497.
3. APHA AWWA, W. E. F. (2005). Standard methods for the examination of water and wastewater. APHA WEF AWWA.
4. Finney DJ. Probit Analysis, 3rd ed. Cambridge University Press, (1971) p. 333.
5. Ac M. The assay of catalases and peroxidases. Methods Biochem Anal. 1954;1:357-408.
6. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase.
7. David M, Richard JS. Glutathione reductase. Methods of Enzymatic Analysis. Bermeyer, Hans, Ulrich, Jr.(Eds.). 1983:258-265.
8. Kim JH, Oh CW, Kang JC. Antioxidant responses, neurotoxicity, and metallothionein gene expression in juvenile Korean rockfish Sebastes schlegelii under dietary lead exposure. Journal of Aquatic Animal Health. 2017 Apr 3;29(2):112-119.