

Research Article



Toxicological Effects of Heavy Metal (Pb^{+2}) on Peroxidase Activity in Freshwater Fish, *Catla catla*

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Abstract | Freshwater ecosystems have been contaminated with a wide range of pollutants. Heavy metals are important contaminants of aquatic system and can cause oxidative stress in fish. Evaluation of antioxidant in fish can reveal metal toxicity in the water ecosystem. Therefore, this experiment was planned to assess the acute affect of lead (Pb^{+2}) on peroxidase (POD) activity in tissues viz. liver, gills, kidney, muscle, heart and brain of fish *Catla catla* exposed for 4-day. After each sampling (24-hr interval) fish were dissected and required organs were obtained. Results showed that the POD activity was augmented in all selected organs of Pb^{+2} exposed fish in relation to control. The POD activity in organs of fish followed the order: brain>liver>gills>kidney>heart>muscle. Regression analysis showed a positive relationship between POD activity and duration of exposure. It was also concluded that antioxidants enzymes can be used as a valuable biomarker of oxidative stress in aquatic animals.

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Introduction

Metals, particularly heavy metals, are significant toxicants of aquatic ecosystem all over the world. Metal pollution has been augmented due to technological development of human society, extensive industrialization and agricultural practices. These metals can persist in water and sediments, and also have ability to amass in aquatic animals like fish (Luoma and Rainbow, 2008). According to Basha and Rani (2003) metals have tendency to alter the physiology and biochemical profile of fish which are important part of ecosystem and also consume as food item.

Lead is a prominent toxicant of water that shows

toxic effects on living being on exposure (Ercal et al., 2001; Pande and Flora, 2002; Patrick, 2006). Aquatic animals can absorb lead from surrounding water, binds to erythrocytes and can passed from blood to body organs (liver, kidney, heart, spleen and muscle) (Meyer et al., 2008). The mechanism of lead toxicity is not understood, it is proved that lead cause oxidative stress by stimulating the generation reactive oxygen species (ROS) (Dewanjee et al., 2013). Aquatic animals possessed defensive system to reduce ROS before the negative effects happen in the cell. This system includes antioxidant enzymes viz. superoxide dismutase, catalase, glutathione peroxidase, glutathione S- transferase and glutathione reductase (Pinto et al., 2003; Tripathi et al., 2006). Superoxide dismutase (SOD) act as a first line of

defense, convert the superoxide radical in to hydrogen peroxide which is further transferred in to oxygen and water by catalase/peroxidase (Jiraungkoorskul et al., 2003; Sanchez et al., 2005).

Recently, antioxidant enzymes as sensitive biomarkers of oxidative stress have gained popularity in ecotoxicology field and are also proved as important indicator of environmental stress before detrimental effects arise in fish, and are significant parameters for evaluating the existence of pollutants in the water (Geoffroy et al., 2004). So, this experiment was performed to analyze the organ and duration specific alteration in peroxidase activity of fish *Catla catla* under acute exposure of lead.

Materials and Methods

Experimental plan

Fresh water fish, *Catla catla* commonly known as “Thaila” were used for this experiment. Fish were obtained from Fish Seed Hatchery, Faisalabad and live shifted to Fish Farm at University of Agriculture, Faisalabad, Pakistan. This experiment was conducted in month of April, 2017. Prior to experiment, fish were kept in cemented tank to acclimatize laboratory conditions for 15-day. After acclimatization, fish were transferred to 100-liter glass aquarium. A group (n=15) of fish were kept in metal exposed medium while control group was kept in metal free water. Chemically pure chloride compound of lead (Pb^{+2}) was used to prepare the solution. The water pH, total hardness and temperature were kept constant as 7.25, 28°C and 225mg L⁻¹, respectively. However, other variables calcium, magnesium, sodium, potassium, total ammonia, carbon dioxide and electrical conductivity were also determined (A.P.H.A., 1998). The 12-hr dark and light period was given to fish.

Acute exposure

The experimental fish *C. catla* was exposed to 96h LC₅₀ concentration (31.25 mgL⁻¹) of lead determined by Batool and Javeed (2015) for 4-day. Sampling was done after 24, 48, 72 and 96h of exposure. At each sampling three fish were sacrificed and required organs viz. gills, liver, kidney, muscle, heart and brain were stored to calculate the peroxidase activity. Three samples replicates were made from each fish organ.

Organ homogenate

Enzyme extract or homogenate was prepared

by adding the phosphate buffer (pH 6.5) in the extracted liver, gills, kidney, muscle, heart and brain by the ratio of 4:1 (w/v). Homogenized material was centrifuged for 15 mint at 10,000 rpm and 4°C by using the refrigerator centrifugal machine. Obtained supernatants were stored at 4°C until further analysis.

Peroxidase assay

The activity of peroxidase was measured according to the method of Civello et al. (1995). Buffer substrate solution was prepared by adding 750 µL of guaiacol into phosphate buffer (47 ml) and mixed well on vortex agitator. After agitation, 0.3 ml of H₂O₂ was added to this solution. Enzyme extract (0.06 ml) and 3ml of buffered substrate solution was taken in a cuvette and absorbance was noted at A₄₇₀ nm by using spectrophotometer. Activity of enzyme is the amount of micromole of a substrate to transform into product by enzyme per mL per minute under standard conditions.

Statistical analysis

Obtained data were analyzed statistically by using the Factorial experiments with three replicates. The value of p<0.05 was considered statistically significant. To find-out relationships between peroxidase activity and exposure duration Regression analyses were also performed.

Results and Discussion

In present work, peroxidase (POD) level was accelerated in liver, gills, kidney, heart, brain and muscles of Pb+2 exposed fish in relation to unstressed fish. It was noted that the POD activity increased significantly with the duration of exposure (Table 1). Organ specific response of POD showed that maximum activity was observed in brain followed by the order: liver>gills>kidney>heart>muscle (Table 2). Regression equation showed significantly positively dependence of the POD activity upon exposure duration with R² values of 0.975, 0.966, 0.962, 0.985, 0.978 and 0.971 for liver, gills, kidney, heart, brain and muscles, respectively (Table 3).

Extensive industrialization and agriculture activities resulted in pollution of water bodies with different toxicants (Vutukuru, 2005). Among these toxicants, metals may also cause negative effects on the variety of aquatic life (Hayat and Javed, 2008). The toxicants such as heavy metals present in the water bodies had

Table 1: Effect of lead on POD activity (Unit/mL) in different tissues of *Catla catla*.

Exposure Duration	Gills		Liver		Kidney		Heart		Brain		Muscle	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
24-hr	0.068±0.003b	0.079±0.003a	1.250±0.020b	1.350±0.020a	0.055±0.002b	0.060±0.002a	0.045±0.001b	0.059±0.020a	1.440±0.025b	1.530±0.026a	0.040±0.010b	0.054±0.020a
48-hr	0.068±0.003b	0.096±0.003a	1.210±0.020b	1.420±0.010a	0.055±0.002b	0.073±0.001a	0.450±0.001b	0.067±0.010a	1.450±0.050b	1.610±0.066a	0.041±0.020b	0.061±0.010a
72-hr	0.068±0.003b	0.148±0.005a	1.210±0.020b	1.600±0.100a	0.056±0.004b	0.090±0.002a	0.045±0.001b	0.077±0.003a	1.450±0.050b	1.800±0.036a	0.040±0.020b	0.069±0.004a
96-hr	0.069±0.004b	0.175±0.004a	1.230±0.010b	1.710±0.010a	0.057±0.004b	0.120±0.003a	0.046±0.001b	0.082±0.003a	1.460±0.036b	1.910±0.002a	0.041±0.020b	0.072±0.004a
Overall Means	0.068±0.001B	0.140±0.040A	1.217±0.012B	1.577±0.146A	0.056±0.001B	0.094±0.024A	0.180±0.234B	0.075±0.008A	1.453±0.006B	1.773±0.152A	0.041±0.001B	0.067±0.006A

Means with similar letters in a single row are statistically similar at $p < 0.05$; ANOVA followed by HSD Tukey test.

Table 2: Tissue-specific response of POD activity (unit/mL) in lead exposed *C. catla*.

Organs	Activity
Liver	1.577±0.146B
Gills	0.140±0.040C
Kidney	0.094±0.024D
Heart	0.075±0.008E
Brain	1.773±0.152A
Muscle	0.067±0.006AF

Means with similar letters in a single column are statistically similar at $p < 0.05$; ANOVA followed by HSD Tukey test.

Table 3: Relationship between exposure duration and POD activity (UmL^{-1}) in various tissues of *C. catla*.

Organs	Regression Equation	SE	R	R ²
Liver	1.21+0.00525**Time(hr)	0.0005922	0.987	0.975
Gills	0.0395+0.00142**Time(hr)	0.0001887	0.982	0.966
Kidney	0.0365+0.000821*Time(hr)	0.0001151	0.981	0.962
Heart	0.0515+0.000329**Time(hr)	0.00002857	0.992	0.985
Brain	1.38+0.00554**Time(hr)	0.0005937	0.989	0.978
Muscle	0.0485+0.000258*Time(hr)	0.00003173	0.985	0.971

SE: Standard Error; r: Multiple Regression Coefficient; Coefficient of Determination; **: Highly significant at $p < 0.01$; *: Significant at $p < 0.05$.

harmful effects at cellular and molecular levels which results in significant modifications in biochemical compositions of the aquatic organisms (Chowdhury et al., 2004). Elevated level of heavy metals may change the activity of antioxidant enzymes. Metals exposure to fish can generate the ROS such as superoxide radical, hydrogen peroxide and hydroxyl radical leading to condition known as “oxidative stress” (Ribeiro et al., 2002; Dautremepuits et al., 2004).

Most of the animals have developed antioxidant enzymes mechanism to scavenge the ROS before the negative effects appeared in the cell (Pinto et al., 2003; Tripathi et al., 2006). Mainly peroxidase plays a significant role in the conversion of H_2O_2 into water and oxygen (Chelikani et al., 2004).

During the present research, Pb^{+2} exposures increased the peroxidase level in gills, liver, kidney, muscle, brain and heart of fish. A similar results were found by Vinodhini and Narayanan (2009) who determined the augmented level of glutathione peroxidase in *Cyprinus carpio* sampled from heavy metals (cadmium, lead, chromium and nickel) contaminated area. This finding clearly shows the protective nature and the adaptive mechanism of cells against free radical induced toxicity. According to Bashir et al. (2018) exposure of CuSO_4 increased the peroxidase level in kidney, liver, brain and gills of *Cirrhina mrigala*. Batool et al. (2018) reported the increased POD activity in kidney and liver of *Cirrhina mrigala* exposed to ZnCl_2 . Shaukat et al. (2018) also noted the increased level of POD in liver and gills of lead exposed *Labeo rohita*. Khan et al. (2015) also noted the significantly increased peroxidase activity in liver and gill of Crucian carp (*Carassius auratus gibelio*) after 96-hr of acute exposure to Pb^{2+} . Raza et al. (2016) reported the significantly increased peroxidase activity in liver and kidney of *Cirrhina mrigala* under lead chloride exposure. According to Atli and Canli (2008) difference in response of the enzymes to toxicants mainly depends upon the tissues, exposure duration and type of metal. Exposure of metal (chromium) increased the peroxidase activity in kidney of gold fish (Velma and Tchounwou, 2010). According to Jastrzebska (2010) lead treated fish (*Cyprinus carpio*)

exhibited significantly augmented peroxidase activity. Present findings are also confirmed by Baysoy et al. (2012) who stated the higher peroxidase level in the hepatic tissues of lead exposed tilapia than that of control fish. Abdel-Khalek et al. (2015) also confirmed significantly enhanced peroxidase level in liver and gills of Zn NPs stressed *Oreochromis niloticus*. Similar findings were observed by Orun et al. (2005) in fish, rainbow trout that were exposed to sodium selenite. Exposure of cadmium increased the level of peroxidase in hepatic and gills of *Labeo rohita* Kumari et al. (2014). According to Rajeshkumar et al. (2013) increased activity of peroxidase was noted in tissues of fish Milk fish (*Chanos chanos*) from polluted sites.

In current study it was noted that peroxidase activity significantly increased with the duration of exposure. In present work higher activity of peroxidase was observed in brain of lead exposed fish. Altaf et al. (2016) also reported significant increase in peroxidase activity in brain of *Labeo rohita* after $MnCl_2$ exposure. Similarly, Vieira et al. (2012) also determined the elevated level of peroxidase in the brain and liver of manganese treated gold fish. Also, Ahmad et al. (2008) noted an increase in the peroxidase activity in three fish species *Dicentrarchus labrax*, *Solea senegalensis* and *Pomatoschistus microps* caught from heavy metals (Cu, Zn, Ni, Pb, and Cr) contaminated two estuaries of the Portuguese coast, Ria de Aveiro and Tejo.

Conclusion

The present study demonstrated that heavy metals exposure greatly influenced the response of antioxidant enzymes in freshwater fish *C. catla*. It was also concluded that the evaluation of antioxidant enzymes could be used as sensitive biomarker in ecotoxicological research.

Author's Contribution

Nosheen executed the research work, Sajid Abdullah was the supervisor and guided the author in planning the research work, Huma Naz helped in writing the article and Khalid Abbas was the member of supervisory committee. He facilitated the author in conducting the research work in his laboratory.

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