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Toxicological Effect of Water-Borne Fe+Zn Mixture on Catalase Activity of *Cirrhina mrigala* and *Labeo rohita*

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ABSTRACT

This study was conducted to assess the effects of the water-borne Fe+Zn mixture on the catalase activity in the different organs of *Cirrhina mrigala* and *Labeo rohita.* 90-day old fingerlings of both fish species were exposed to 1/2nd and 1/5th of their respective 96-hrs LC_{50} concentrations of Fe+Zn mixture, separately, at constant temperature (28^{0}C), pH (7.5), and total hardness (225mgL^{-1}). Fish sampling from each treatment was done after 7, 14, 21, and 28 days of Fe+Zn mixture exposure and their organs viz. liver, kidney, gills, and muscles extracted for catalase enzyme assay. The maximum catalase activity in *Cirrhina mrigala* was observed at 1⁄ 5th of Fe+Zn mixture LC_{50} exposure as 996.87±102.23UmL^{-1}, while in *Labeo rohita* it was 1013.43±103.50 UmL^{-1} at 1⁄ 2nd of LC_{50} concentration. A significant increase in the catalase activity was recorded at 7th day of exposure as 1083.66±7.29 and 1091.08±22.15 UmL^{-1} in *Cirrhina mrigala* and *Labeo rohita*, respectively while it was significantly lower at 28th day of Fe+Zn mixture exposure. Among the organs, the catalase activity varied significantly as liver > kidney > muscles > gills in *Cirrhina mrigala* and *Labeo rohita*. Significantly higher catalase activity was recorded in *Labeo rohita* as compared to *Cirrhina mrigala.*

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INTRODUCTION

Among aquatic contaminants, heavy metals are the most lethal pollutants that enter the food chain through fish. The wide range of metallic ion pollutants has hazardous impacts on aquatic animals as some metals cause disturbance in natural processes of transformation by accumulating in various organs, particularlythe in liver and kidney. Heavy metals can disturb the integrity of biochemical mechanisms, physiological activities and cause deleterious effects on fish (Ruas *et al*., 2008).

In the aquatic ecosystems, metals are mostly existed in the form of mixtures due to different point and non-point sources of their discharge (Lang *et al*., 2002). The effects of metals in their mixture form are more severe to fish as compared to the individual form because in mixture form, some metals have synergistic or antagonistic impacts that may increase or decrease their toxicity to the affected organism (Otitoloju, 2003). The toxicity of metals varied from species to species or even metal to metal (Tchounwou *et al*., 2008). At higher exposure concentration, metals produce reactive oxygen species that cause the oxidation of proteins, lipid and amino acid and can even cause cell death (Oner-Muazzez *et al*., 2009). For different physiological processes, iron (Fe) plays an important role. It binds to the haemoglobine, transferrin and iron-containing enzymes and affect their functioning (Kontoghiorghes et al., 2020). Iron can bind the surface of the gill and oxidize to insoluble iron. This insoluble form of iron covers the gill lamellae and cause epithelial damage and disturbance in respiratory processes (Park *et*

al., 2007). Excessive uptake of iron causes cellular injury due to iron catalysis of ROS formation through the Fenton reaction (Oteiza, 2012).

Zinc (Zn) is a metallothionein inducer and an important co-factor of superoxide dismutase (Pilon, 2011). However, zinc has inhibitory effects on catalase activity, which lead to oxidative stress. Catalase acts as an antioxidant enzyme that provides the first line of defense against oxidative stress and helps in redox regulation in different body tissues (Faheem *et al*., 2012). It converts hydrogen peroxide into water and oxygen. Aquatic pollutants are affecting the antioxidant defense system of fish by either enhancing the production of reactive oxygen species or decreasing the activity of antioxidants enzymes. Catalase activity is now widely used as a sensitive biomarker of oxidative stress (Sarwar *et al.,* 2014). The fish species *Labeo rohita* and *Cirrhina mrigala* are important freshwater fishes that are widely consumed in Pakistan because of their better meat quality (Ahmad *et al*., 2012). Consumption of metal contaminated fish may lead to metal accumulation in the human body and eventually affect their health (Bahnasawy *et al*., 2009). Fish serve as a bio-monitoring tool to assess levels of pollution in natural aquatic bodies. Therefore, the present research was done to measure the effects of water-borne Fe+Zn mixture on catalase activity in different organs of *Cirrhina mrigala* and *Labeo rohita.*

MATERIAL AND METHODS

This research work was conducted in the laboratories of Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. The fish, *Cirrhina mrigala* and *Labeo rohita* were procured from the Fish Seed Hatchery and brought to the laboratory for acclimatization in cemented tanks for fifteen days. After the acclimation period, the healthy fingerlings of both fish species having similar average weights and lengths will be selected for these experiments. Chemically pure chloride compounds of iron ($FeCl_2.H_2O$) and zinc ($ZnCl_2$) will be dissolved in deionized water, separately, to prepare the stock solutions. The stock solutions were mixed on an ion equivalence basis to prepare Fe+Zn mixture stock solutions. The 90 days old three fish groups (n=10) were transferred to glass aquaria of 50L water capacity to measure the effect of Fe+Zn mixture on catalase activity in the selected tissue viz. liver, kidney, gills, and muscles of *Cirrhina mrigala* and *Labeo rohita*, separately, at constant temperature (28°C), pH (7.5) and total hardness (225mgL^{-1}). Each was conducted with three replications for each test dose along with the control group (un-stressed). Both the fish species were exposed to a metals mixture for 30 days with the following sub-lethal concentrations of their respective 96-hr LC_{50} values of Fe+Zn as determined by Naz (2013):

Fish species	Treatments	Sub-lethal concentrations (mgL^{-1})
Cirrhina mrigala	1/2nd	38.08±0.30
	1/5th	15.23±0.12
Labeo rohita	1/2nd	53.58±0.30
	1/5th	18.10±0.12

Fish were sampled randomly after 7, 14, 21, and 28 days of metals mixture exposure. After each time interval, the kidney, gills, liver, and muscles sampled were isolated and preserved at -4°C for the estimation of enzyme assay. The physical and chemical characteristics viz. pH, dissolved oxygen, carbon dioxide, total hardness, calcium, magnesium, and total ammonia are monitored twice daily by following the methods of A.P.H.A. (2012).

Enzyme Assay

Red blood cells are removed from the kidney, gills, liver, and muscles by rinsing these organs with phosphate buffer of pH 6.5 (0.2M) and homogenized in cold buffer (1:4w/v) with a pestle and mortar. After homogenization, the organs homogenate will be centrifuged for 15 minutes at 10,000rpm at 4ºC. After the centrifugation process, clear supernatant was preserved at -4ºC for enzyme assay while residue was discarded. For the determination of catalase activity, the sample was subjected to enzyme assay by following the methods described by the method of Chance and Mehaly (1977).

Required reagents for enzyme assay

1. 60mM sodium phosphate ($NaSO_4$) buffer, pH 7.0
2. 10mM H_2O_2

Preparation of 60mM phosphate buffer, pH 7.0

0.224g $Na.H_2.PO_4$ and 0.1632g $Na_2.H_2.PO_4$ was taken in a flask and dissolved with distilled water. Then volume was raised to 50mL and adjusted the pH was 7.0.

Preparation of buffer substrate solution

Buffer substrate solution of 10mM of H_2O_2 was prepared in 160mM phosphate buffer by dissolving 0.442mM H_2O_2

in 60mM phosphate buffer. The reaction mixture of 2mL contains:

Buffered substrate solution	1.95mL
Enzymes extract	0.05mL

Blank 66mM phosphate buffer was used as a blank.

Procedure

The absorption of the blank solution was recorded first at 240nm in a spectrophotometer. Then a cuvette containing buffered substrate solution was introduced into the spectrophotometer and initiation of the reaction was occurred by adding 0.05mL of enzyme extract the initiation of reaction has occurred. The reaction time is 3 minutes and noted the absorbance after an interval of 1 minute.

Calculations

$$\text{Activity (Units/mL)} = \frac{\text{AA/min} \times \text{dilution} \times 2\text{mL}}{0.04\text{mM}^{-1}\ \text{cm}^{-1} \times 0.05\text{mL}}$$

RESULTS

Change in catalase activity in *Cirrhina mrigala*

Show variation in CAT activity after exposure of Fe+Zn mixture for 28 days in the organs of Cirrhina mrigala express in Table 1. Analysis of variance shows statistically significant differences between duration of exposure, among treatments and organs for CAT activity Cirrhina mrigala. Significantly higher activity of CAT was recorded as 996.87±6.90**UmL^{-1} in** the 1/5th of LC_{50} exposed Cirrhina mrigala while, the activity of CAT in control fish was lower significantly as 970.56±16.49**UmL^{-1},** among the treatments control, 1/2nd and l/5th**.** During the different periods of metals mixture exposure, the activity of CAT was a maximum 1083.66±7.29UmL^{-1}, tend to decrease 855+7.87**UmL**$^{-1}$ on 28th day of metals exposure. The order followed by CAT activity in the organs in all exposure duration was as follows: 7 > 14 > 21 > 28-day. However, at the l/5th of LC_{50} *of* Fe+Zn mixture exposure the CAT activity followed the order: liver > kidney > muscles > gills.

Table 1. Catalase activity (UmL^{-1}) in tissues of *Cirrhina mrigala*, after chronic exposure to Fe+Zn mixture.

	Organ				Overall means±SD
	Kidney	Gills	Liver	Muscles	
Dose Dependent					
1/2nd	964.25±97.34d	983.25±100.40c	1003.00±116.12a	987.00±99.20b	984.37±15.91b
1/5th	996.50±99.92b	989.00±99.32d	1010.50±110.50a	991.50±99.70c	996.87±19.60a
control	975.25±89.43b	958.50±12.21b	991.75±101.35a	956.75±99.29d	970.56±16.40c
means±SD	978.66±16.39b	976.91±16.20c	1001.75±9.43a	978.41±18.89b	
Time Dependent					
7	1082.00±2.30b	1068.00±14.79d	1107.66±24.00a	1076.66±3.21c	1083.66±7.29a
14	959.66±13.61b	976.66±57.29d	1064.66±4.40a	1023.66±57.81c	1037.41±18.80b
21	975.33±4.04a	960.33±4.61d	973.66±6.50b	965.00±75.00c	968.58±1.433c
28	901.83±82.78b	887.66±89.38d	907.83±88.94a	893.50±85.64c	855.00±7.87d
means±SD	978.66±16.39b	976.91±16.20c	1001.75±9.43a	978.41±18.89b	

Duration	Treatment			
	Control	1/2nd	1/5th	means±SD
7	1073.50±15.28c	1087.25±22.09b	1090.25±21.86a	1083.66±7.29a
16	1017.75±58.93c	1031.75±48.23b	1062.75±6.44a	1037.41±18.80b
21	968.75±17.67b	966.75±8.80c	970.25±7.80a	968.58±1.433c
28	845.00±21.30c	855.75±5.90b	864.25±4.34a	855.00±7.87d
means±SD	976.25±97.40c	985.37±99.46b	996.87±102.23a	

Change in catalase activity in *Labeo rohita*

The CAT activity in the organs viz. kidney, gills, liver, and muscles of *Labeo rohita,* under different treatments of Fe+Zn mixture, is presented in Table 2. The activity of CAT changed significantly ($p<0.05$) in organs of fish in all treatments and exhibited maximum CAT activity in fish treated with l/2nd of LC_{50} of Fe+Zn mixture. Among different treatments, CAT activity was maximum in l/2nd of LC_{50} as 1013.43±22.6UmL^{-1} followed by l/5th (1003.37±5.07UmL^{-1}) and control (997.25+3.25UmL^{-1}).

Among the organs, comparisons of means indicate that CAT activity followed the trend: liver > kidney > muscles > gills. In the *Labeo rohita,* CAT activity declined with an increase in exposure duration. CAT activity was maximum on day 7th as 1091.08±22.15UmL^{-1} and on day 28, it was minimum as 878.66UmL^{-1}. During all the exposure duration, significantly higher activity was recorded in the liver as 1015.58±18.28UmL^{-1}, kidney (1006.91±11.24UmL^{-1}), muscles (1004.91UmL^{-1}), and gills (994.41±12.25UmL^{-1}). Figure 1 shows the comparison of the change in catalase activity in the tissues of *C. mrigala* and *L. rohita* after exposure to the Fe+Zn mixture.

Physico-chemical Analyses

Physico-chemical parameters viz. temperature, pH, total hardness, total ammonia, DO, CO2, Ca, and Mg were recorded during the experimental trials and presented in table 3 for *Cirrhina mrigala* experimental media and Labeo rohita in table 4 for media.

Table 2. Catalase activity (UmL^{-1}) in tissues of *Labeo rohita*, after chronic exposure to Fe+Zn mixture.

	Organ				means±SD
	Kidney	Gills	Liver	Muscles	
Dose Dependent					
1/2nd	1017.50±110.68b	982.00±74.51d	1036.50±129.47a	1014.75±106.70c	1013.43±22.60a
1/5th	1005.00±102.21b	996.75±102.62d	1008.75±92.70a	1003.00±94.25c	1003.37±5.01b
control	996.75±2.22b	994.75±2.30d	1002.00±2.75a	995.50±2.57c	997.25±3.27c
means±SD	1006.91±11.24b	994.41±12.25d	1015.58±18.28a	1004.91±10.50c	
Time Dependent					
7	1097.00±33.04b	1062.00±25.98d	1115.33±66.58a	1090.00±28.16c	1091.08±22.15a
14	1074.66±5.77b	1055.00±17.34d	1077.33±3.78a	1068.33±7.63c	1068.83±9.96 b
21	978.00±8.54b	959.66±33.85d	982.66±5.77a	970.66±5.03c	972.74±10.02 c
28	881.00±5.19b	868.33±8.02d	887.66±1.15a	877.66±2.88c	878.66±8.04 d
means±SD	1006.91±11.24b	994.41±12.25d	1015.58±18.28a	1004.91±10.50c	

Duration	Treatment			
	Control	1/2nd	1/5th	means±SD
7	1073.25±2.46c	1120.25±2.59a	1079.75±2.71b	1091.08±22.15a
16	1065.00±2.60c	1067.00±2.52b	1081.00±2.65a	1068.83±9.96 b
21	973.25±2.42c	983.25±2.40a	981.25±2.42b	972.74±10.02 c
28	867.50±2.36c	883.25±2.30a	877.50±2.26 b	878.66±8.04 d
means±SD	997.25±91.80c	1013.43±103.50a	1005.37±96.76b	

Table 3. Mean value of physic-chemical parameters determined during Fe+Zn mixture exposure to *Cirrhina mrigala*.

Treatment	Duration (days)	Temperature (ºC)	pH	Total Hardness (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Total Ammonia (mgL^{-1})
1/2nd	7	28.00±0.02	8.25±0.02	288.84±1.73	5.20±0.51	0.66±0.05	25.90±0.98	54.55±0.52	0.88±1.38
	14	29.08±0.04	8.21±0.01	290.76±1.62	5.16±0.50	0.73±0.07	25.93±1.21	56.51±0.63	0.90±1.48
	21	28.00±0.02	8.26±0.02	292.66±1.61	5.10±0.41	0.79±0.09	25.95±1.40	58.45±0.73	0.92±1.56
	28	27.25±0.03	8.29±0.03	296.75±1.65	5.15±0.45	0.87±0.08	27.89±0.97	60.5±0.75	0.97±1.74
Mean±S.D.		28.35±1.31	8.19±0.54	292.25±3.37	5.15±0.04	0.76±0.08	26.41±0.98	57.50±2.55	0.91±0.03
1/5th	7	28.15±0.03	8.07±0.03	268.30±1.44	5.34±0.37	0.40±0.04	20.49±0.56	51.06±0.51	0.86±1.37
	14	27.70±0.04	9.03±0.02	268.21±1.55	5.31±0.35	0.42±0.05	21.46±0.65	52.02±0.23	0.93±1.39
	21	28.15±0.03	8.08±0.01	268.01±1.54	5.26±0.32	0.45±0.07	22.41±0.56	54.96±0.46	0.94±1.45
	28	27.40±0.02	8.11±0.03	268.21±1.72	5.30±0.34	0.47±0.09	25.45±0.54	55.01±0.49	0.99±1.74
Mean±S.D.		28.41±1.17	8.41±0.54	268.20±0.08	5.30±0.03	0.43±0.03	22.45±2.14	53.26±2.02	0.93±0.05
Control	7	28.25±0.04	8.03±0.02	251.48±1.34	5.38±0.23	0.41±0.04	21.56±0.50	49.65±0.78	0.87±1.45
	14	29.60±0.05	8.07±0.03	252.58±1.44	6.31±0.52	1.45±0.09	21.86±0.60	49.72±0.43	1.82±1.48
	21	28.25±0.03	8.04±0.03	261.66±1.33	5.59±0.51	0.42±0.07	21.98±0.71	49.97±0.64	0.88±1.41
	28	27.50±0.04	7.99±0.01	271.57±1.71	5.34±0.27	0.37±0.05	22.25±0.30	51.61±0.83	0.92±1.32
Mean±S.D.		28.45±1.06	8.37±0.61	259.32±9.35	5.47±0.18	0.75±0.61	21.91±0.28	50.23±0.92	1.21±0.53

Table 4. Mean value of physic-chemical parameters determined during Fe+Zn mixture exposure to *Labeo rohita*.

Treatment	Duration (days)	Temperature (ºC)	pH	Total Hardness (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})	Total Ammonia (mgL^{-1})
1/2nd	7	28.00±0.04	8.25±0.04	282.84±1.84	5.20±0.34	0.66±0.13	25.93±1.94	54.55±0.42	0.88±1.32
	14	29.08±0.09	9.21±0.03	282.76±1.63	5.16±0.43	0.73±0.16	25.90±1.64	54.51±0.51	0.90±1.64
	21	28.00±0.04	8.26±0.02	282.66±1.52	5.10±0.52	0.79±0.18	25.85±0.56	54.45±0.63	0.92±1.74
	28	27.25±0.05	8.29±0.03	282.75±1.41	5.15±0.21	0.87±0.22	25.89±0.96	54.50±0.73	0.97±1.85
Mean±S.D.		28.35±1.31	8.59±0.54	282.75±0.07	5.15±0.04	0.76±0.08	25.89±0.03	54.50±0.04	0.91±0.03
1/5th	7	28.15±0.09	8.07±0.01	268.30±1.63	5.34±0.32	0.40±0.11	22.49±1.02	53.06±0.32	0.86±1.23
	14	27.70±0.07	9.03±0.03	268.21±1.52	5.31±0.23	0.42±0.14	22.46±0.56	53.02±0.42	0.93±1.64
	21	28.15±0.08	8.08±0.02	268.10±1.42	5.26±0.42	0.45±0.16	22.41±0.76	52.96±0.52	0.94±1.64
	28	17.40±0.06	8.11±0.01	268.20±1.31	5.30±0.26	0.47±0.21	22.45±1.67	53.01±0.62	0.99±1.75
Mean±S.D.		28.41±1.17	8.41±0.54	268.20±0.08	5.30±0.03	0.43±0.03	22.45±9.99	53.01±0.04	0.93±0.05
Control	7	28.25±0.05	8.03±0.01	251.48±1.87	5.38±0.42	0.41±0.24	21.30±0.44	49.65±0.74	0.87±1.45
	14	29.06±0.03	9.07±0.03	251.58±1.89	6.31±0.53	0.45±0.17	21.26±0.64	49.62±0.64	1.82±1.78
	21	28.25±0.06	8.04±0.02	251.66±1.74	5.59±0.32	0.42±0.19	21.23±1.02	49.57±0.81	0.88±1.93
	28	27.50±0.07	7.99±0.03	251.57±1.64	5.34±0.23	0.37±0.17	21.22±1.37	49.61±0.64	0.92±84
Mean±S.D.		28.45±1.06	8.37±0.61	251.57±0.07	5.47±0.18	0.75±0.61	21.25±0.04	49.61±0.03	1.21±0.53

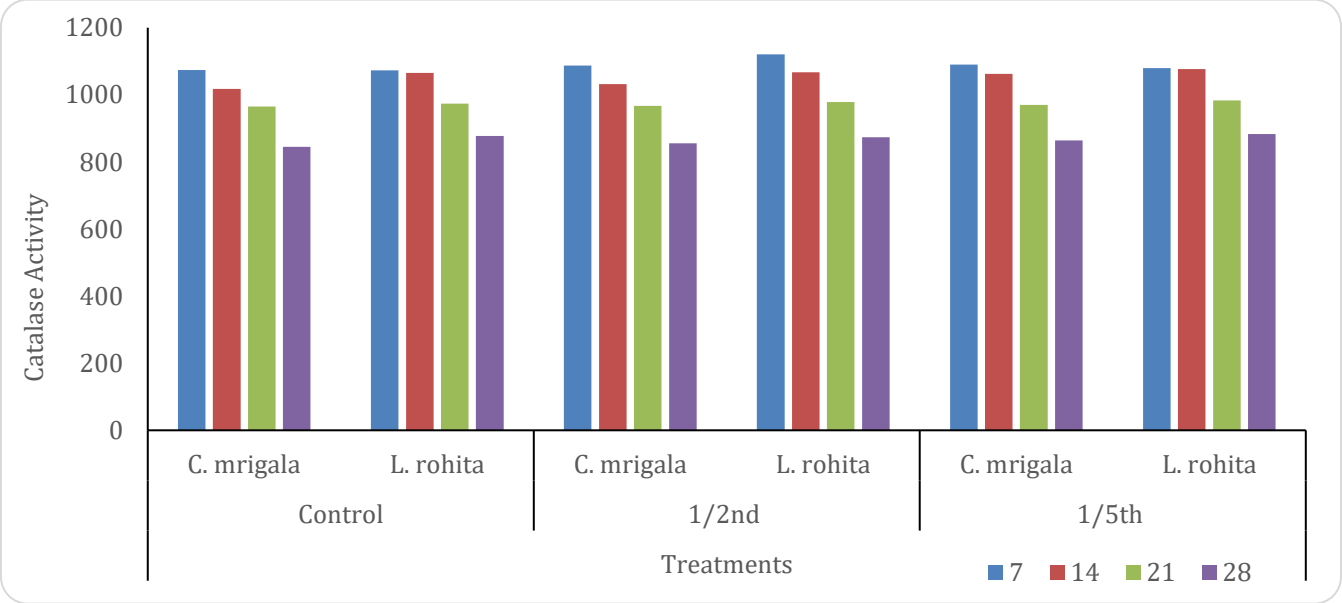

Figure 1. Comparison of catalase activity in Fe+Zn exposed *C. mrigala* and *L. rohita.*

DISCUSSION

The present research work was designed to compare catalase (CAT) activity in kidneys, gills, liver, and muscles of normal and Fe+Zn exposed *Labeo rohita* and *Cirrhina mrigala*. Metals can cause oxidative damage to the cell due to the higher production of reactive oxygen species (ROS) by Fenton- and Haber-Weiss type mechanisms (Baysoy *et al*., 2012). Heavy metals they can generate oxidative stress in fish species because of their strong oxidative nature. CAT is one of the important antioxidant enzymes, which plays an important role in the cellular defenses by the decomposition of H_2O_2 into water and oxygen and prevents the tissues from oxidative damage (Faheem *et al*., 2012). The present research work showed that the CAT activity was higher in the liver of the fishes as compared to the other organs. The activity of CAT was decreasing in order of gill < muscle < kidney < liver. The results showed higher activity of catalase in the liver as compared to the gills and muscles. The results were also supported by Hidalgo *et al*., (2002) and Gul *et al*. (2004) high CAT activity was determined in liver tissue as

compared to other body tissues, which is in agreement with other literature.

The conclusions of this research showed higher activity of CAT in the liver of fish sampled from the contaminated sites. The present study revealed that the activity of CAT was increased in order as follows liver>kidney>gills>muscles. The same decreasing trend was reported in the studies of Hidalgo *et al.* (2002) who worked on the oxidative stress in rainbow trout and results showed increased activity of CAT in the liver as compared to the other organs of fish. The results of the present study revealed that CAT activity in the gills is lower than in the liver, kidney, and muscles. These results were supported by Patil *et al*., (2013) who observed the impact of malathion exposure on anti-oxidative enzymes of *L. rohita.* The fish gills are in direct contact with the external environment; therefore, the fluctuations are assessed to be noticeable. Results showed lower activity of CAT enzyme. The results of the present study were also supported by the Vutukuru *et al*., (2006) catalase activity increased during experimental periods and are probably a response to toxicant stress increase ROS generation who studied the effect of the CAT in various body tissues. Gills were the sensitive organ, exhibiting reduced CAT activity. The reduced activity of gills as compared to the other tissues. The reduced activity of gills was also supported by Gulzar *et al*., (2006) who evaluated the CAT activity in gill and liver tissues of *Oreochromis niloticus* treated with different concentrations of Ag and Zn.

This work was supported by Vinodhini and Narayanan (2008) who suggested that the liver tissues in fish are more often recommended as an environmental indicator of water pollution than any other organs. The toxicants cause a disturbance in the physiological state of the fish, which affects the enzyme activity. It then causes distortions in the cell organelles, which may lead to the elevation in the activity of various enzymes. Liver enzymes are considered more sensitive to mental stress as compared to the kidney. Also, the liver is known to be a strong organ towards oxidative stress than the other organs and has highest antioxidant enzyme activities. This could be related to the fact that the liver is the site of multiple oxidative reactions and maximal free radical generation (Gul *et al*., 2004).

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