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TOXICOLOGICAL EFFECTS OF A BINARY MIXTURE OF METALS (Fe+Mn) ON THE OXIDATIVE STRESS OF MAJOR CARPS

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ABSTRACT

Oxidative stress caused by various pollutants in water can be overcome by different mechanisms in aquatic organisms. Antioxidant enzymes play major role to minimize the hazardous effect of heavy metals in fish. Superoxide dismutase is one of the antioxidant enzyme that converts the superoxide radical into hydrogen peroxide (H_2O_2) and thus minimize the stress caused by free radicals. Therefore, the aim of this research was to evaluate the activity of superoxide dismutase (SOD) in the organs (liver, kidney, gills and muscles) of *Catla catla* and *Cirrhina mrigala* exposed to 1/3rd and 1/4th concentrations of 96-hr LC_{50} of Fe+Mn. These experiments were conducted in glass aquaria with three replications of each concentration for 1 month in controlled laboratory conditions. SOD activity was significantly increased in liver and gills of both fish species compared to control group. Maximum SOD activity in the Fe+Mn mixture exposed *Catla catla* was measured as 46.55±7.58 and 43.69±5.34 UmL^{-1} in the liver and gills, respectively, at 1/4th concentration of 96-hr LC_{50} while in control fish the activity of SOD was recorded as 33.36±6.72 and 34.16±3.19 UmL^{-1}, respectively. Similarly, SOD activity increased with decrease in concentration in *Cirrhina mrigala*. Maximum activity of enzyme (52.30±7.33 UmL^{-1}) in *Cirrhina mrigala* was observed in the liver at 1/4th concentration of 96-hr LC_{50} compared to control in which the activity of enzyme was recorded as 46.95±3.93 UmL^{-1}. The SOD activity in all the metals mixture treated and un-treated fish groups were found lower in muscles than that of other organs.

Keywords: antioxidant enzyme, *Catla catla, Cirrhina mrigala,* heavy metals, superoxide dismutase

INTRODUCTION

In South Asian countries, particularly in Pakistan, India and Bangladesh, Carp culture is very popular practice and considered as main production system of aquaculture (Reddy *et al*., 2002). Among carp species, *Catla catla*, *Cirrhina mrigala* and *Labeo rohita* are preferred in Asian countries (Rahman *et al*., 2006). Variety of toxic heavy metals are reported to be present in rivers and reservoirs all over the world which affects to the aquatic organisms living in the recipient environment (Farombi *et al*., 2007). Effects of heavy metals on aquatic organisms can be measured by change in biochemical parameters in the organs of the fish that respond specifically to the type and degree of contamination (Barhoumi *et al*., 2012). As metals in the aquatic environment are present in the form of mixtures, their persistence above tolerance limits in fish may produce histological and biochemical changes in them (Bu-Olayan and Thomas, 2004).

Iron (Fe), an essential metal which is involved in the oxygen transport chain, DNA synthesis, immune functions and respiratory chain reactions. It is one of the important metal to be mined all over the world. Elevated levels of Fe cause toxicity to the organisms either by external exposure from diet and water or by internal breakdown of Fe in homeostatic regulations (Bury *et al*., 2011). Iron in the form of Fe^{+2} is more toxic to aquatic organisms as compare to Fe^{+3} (Decker and Menendez, 2000). Hydroxyl radicals (OH^-, OH) are very reactive molecules, they cause damage to nucleic acids and peroxidation of lipid membranes (Li *et al*., 2009). Disruption due to hydroxyl radicals in the structure and activity of macromolecules is so severe, that it may lead towards tissue injuries (Brewer, 2010).

Manganese (Mn) acts as a cofactor for superoxide dismutase (SOD) and form Mn-SOD that provide resistance against oxidative stress.

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However, elevated levels of Mn especially in brain can cause neurotoxicity. It can cause oxidative stress as well as disturbance in the metabolic pathways of neurotransmitters (Miele *et al*., 2000; Stokes *et al*., 2000). Mn deficiency is rare but toxicity due to its over exposure is more prevalent (Crossgrove and Zheng, 2004) Extent of toxicity of Mn depends on the species, life stage of fish and surrounding water quality (Fish, 2009). Heavy metals contamination significantly hampers the reproductive performances of fish (Yamaguchi *et al.,* 2007). Investigations have reported several reproductive compromises including reduced GSI, fecundity, hatching rate, fertilization success, abnormal shape of reproductive organs, and finally overall reproductive success in response to a variety of heavy metals (Garriz *et al*., 2019; Vajargah *et al.,* 2020; Yan *et al.,* 2020).

Toxicity of metals increased in mixture form due to their interaction with each other as well as the metallic ions that compete for their binding sites (Otitoloju, 2003). Concentration, composition and exposure period of metals mixtures are the major factors, contributing for their toxicity in fish (Javed, 2012). In adverse environmental conditions, the role of antioxidant enzymes especially, SOD gained much attention because it indicates the physiological and biochemical changes in fish due to accumulation of heavy metals (Elia *et al*., 2003; Barata *et al.,* 2005). Therefore, present research aimed to study the changes in SOD activity in the selected organs of *Catla catla* and *Cirrhina mrigala* exposed to Fe+Mn mixture.

MATERIALS AND METHODS

The proposed experimental work was conducted under controlled laboratory conditions at the Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad. After 90 days *Cirrhina mrigala* and *Catla catla* were brought to the wet laboratory for acclimation, obtained from the Fish Seed Hatchery, Faisalabad. In cemented tanks fish were led to adapt for the period of 2 weeks before start of the experiment and were fed with pelleted feed two times a day. After 2 weeks, fish having same lengths and weight were selected for further studies.

Metal solution

For the preparation of metals stock solution, pure chloride compound of manganese and iron was dissolved in 1000mL de-ionized water according to their molecular weights in salt form and then these stock solutions were mixed in 1:1 ratio to make the stock solution of Mn+Fe solution.

Treatments

Catla catla and *Cirrhina mrigala* were exposed to the following sub-lethal concentrations of Fe^{+} and Mn mixture as per determined by Naz (2013) for a period of 30 days by using a static water system with continuous aeration under controlled laboratory conditions:

Fish species	Treatments	Sub-lethal concentrations (mgL^{-1})
Catla catla	$1/3^{rd}$ $1/4^{th}$	24.71±0.19 18.53±0.14
Cirrhina mrigala	$1/3^{rd}$ $1/4^{th}$	24.13±0.15 18.10±0.11

Enzyme assay for superoxide dismutase

The antioxidant activity was measured by isolating the fish organs viz. gills, kidney, muscles and liver after the 30 days of exposure to Fe+Mn mixture. The antioxidant activity in the isolated fish organs were compared with the control group with 3 replications for each concentration. The superoxide dismutase activity was determined by following the enzyme assay of Giannopolitis and Ries (1977) and enzyme activity was assessed by determining its ability to inhibit the photo-reduction of nitroblue tetrazole (NBT).

Preparation of enzyme extract

The isolated organs were rinsed with phosphate buffer of pH 6.5 (0.2 M) and then blended in cold buffer (1:4 w/v) for removing of red blood cells. Organ homogenates were centrifuged for 15 minutes at 10,000 rpm and 4ºC. Clear supernatants were stored for enzyme assay at -80 ºC and residue was discarded.

Preparation of 0.067 mM phosphate buffer, pH 7.8

0.14g KH_2PO_4 was added in 0.74g $K_2H\ PO_4$ in flask and volume was made upto 80ml by distilled water.

Preparation of EDTA/NaCN solution

0.16g EDTA was taken in a flask and 0.08mg NaCN was added in it and volume was made upto 5.4ml with distilled water.

Preparation of riboflavin solution

0.06mg riboflavin was taken in a flask and its volume was made upto 1.3ml with distilled water. It was stored in cold dark bottle.

Preparation of NBT solution

3.23mg NBT was taken in a flask and with distilled water its volume was made upto 2.64ml. It was stored in cold dark bottle.

Procedure

First of all for blank run 1 ml of buffer was taken in a cuvette and placed into the spectrophotometer and reading was noted. After that spectrophotometer was adjusted at zero at A_{560} nm. 5-6 cuvettes were placed in a light box (with an internally mounted light bulb of 30 Watt) and 1ml of buffer was added to each cuvette. Then 0.05 ml enzyme extract and 0.016ml of riboflavin was added in each cuvette and all the cuvettes were incubated in light box for 12 minutes. After that all cuvettes were transferred to the spectrophotometer where 0.067ml of EDTA/ NaCN solution and 0.033 ml of NBT was added to the illuminated reaction mixture. After 20 seconds of reaction, the absorbance was measured. By measuring the % age inhibition of NBT, activity of superoxide dismutase was determined.

$$(\%\ \text{age inhibition}) = \frac{\text{Blank (Abs) - Sample (Abs)}}{\text{Blank (Abs)}} \times 100$$

RESULTS

The laboratory experiments were conducted to determine the activity of superoxide dismutase (SOD) in the selected organs of *Catla catla* and *Cirrhina mrigala* exposed to sub-lethal concentrations of Fe+Mn mixture. The physico-chemical parameters viz., pH, water temperature, total hardness, carbon dioxide, calcium, dissolved oxygen, magnesium and total ammonia were also monitored on 12 hourly basis.

Superoxide dismutase enzyme activity in *Catla catla*

After exposure to various sub-lethal concentrations of Fe+Mn mixture to the *Catla catla*, the superoxide dismutase (SOD) activity was measured in the muscles, liver, gills and kidney of *Catla catla* and presented in (Table 1). SOD activity increased significantly in the Fe+Mn mixture treated fish as compared to control. Among the treatments, lower activity of SOD was measured in control fish group as 28.47±7.87 UmL^{-1}, while significantly higher activity (43.01±2.89 UmL^{-1}) was recorded in *Catla catla* exposed to 1/4th concentration of LC_{50}. In the control fish group, maximum SOD activity was observed in gills as 34.16±3.19 UmL^{-1} followed by liver, kidney and muscles. However, at 1/4th of LC_{50} exposure maximum activity was observed in liver (46.55±7.58 UmL^{-1}) and minimum in muscles as 39.65±5.26 UmL^{-1}, while at 1/3rd of LC_{50} of Fe+Mn exposure, the SOD activity followed the order: gills>kidney> liver>muscle. At different exposure durations, SOD activity increased with increasing exposure duration up to 15 days after which it started decreasing. Maximum SOD activity was observed at day 15 while significantly minimum was observed at day 30. Significantly higher activity of SOD in gills was observed at day 10 with the mean value of 43.62±5.87 UmL^{-1} while the maximum activity of liver was measured at day 15 as 49.00±11.74 UmL^{-1}. The least SOD activity was recorded in muscles at all durations except day 1. The overall pattern of durations for SOD activity followed the order 15>10>5>30-day. At all durations, maximum SOD activity was observed in 1/4th of LC_{50} exposed fish followed by 1/3rd and control with mean values of 43.01±4.53 UmL^{-1}, 39.86±8.34 and 28.47±3.69 UmL^{-1}, respectively.

Table 1. Superoxide dismutase activity (UmL^{-1}) in the selected organs of *Catla catla*, after chronic exposure to Fe+Mn mixture

	Duration	Organs				Overall Means
		Liver	Kidney	Gills	Muscles	
Control	05	32.56±1.42	29.68±1.27	36.21±1.51	18.45±0.60	29.23±7.66b
	10	40.58±2.61	31.70±1.22	37.15±1.56	22.50±0.62	32.99±7.89a
	15	35.61±1.49	26.03±0.72	33.19±1.47	15.44±0.53	27.57±9.05c
	30	24.55±0.90	29.64±0.83	30.11±1.36	12.11±0.41	24.10±8.39d
	Overall Means	33.36±6.72b	29.26±2.36c	34.16±3.19a	17.12±4.42d	--
1/4th	05	40.35±1.97	46.87±2.72	37.85±1.89	43.37±2.68	42.11±3.89b
	10	43.36±2.19	40.11±2.24	45.11±2.63	37.63±1.28	41.56±3.34c
	15	57.56±3.41	45.56±2.67	50.35±3.26	44.44±2.52	49.48±5.97a
	30	44.93±2.24	36.10±1.53	41.46±2.11	33.17±1.21	38.92±5.28d
	Overall Means	46.55±7.58a	42.16±4.99c	43.69±5.34b	39.65±5.26d	--
1/3rd	05	35.12±1.71	38.85±1.46	25.56±0.79	40.35±2.03	34.97±6.65c
	10	41.61±2.19	44.36±2.38	48.61±2.51	48.37±2.78	45.74±3.37b
	15	53.82±3.27	50.74±3.13	55.56±3.51	31.94±1.07	48.02±10.90a
	30	26.34±0.81	34.63±1.28	40.49±2.06	21.41±0.76	30.72±8.50d
	Overall Means	39.22±11.57c	42.14±6.98b	42.56±12.90a	35.52±11.55d	--

Superoxide dismutase enzyme activity in *Cirrhina mrigala*

SOD activity varied significantly in different organs and treatments in Fe+Mn mixture exposed *Cirrhina mrigala* (Table 2). Analysis of variance reveals statistically significant variability among the organs, at treatments and durations for which fish were exposed to Fe+Mn mixture. The SOD activity in the fish organs at three treatment showed significant variations with the higher mean activity in 1/4th of LC_{50} exposed fish. Among the treatments, SOD activity followed the order: 1/4th (44.00±9.04 UmL^{-1}) > control (40.96±5.24 UmL^{-1}) > 1/3rd (39.74±6.60 UmL^{-1}). Comparisons of means show that significantly higher SOD activity was observed in liver with the mean value of 49.26±2.74 UmL^{-1} followed by gills, kidney, and muscles as 43.54±4.92 UmL^{-1}, 40.62±2.38 and 32.86±1.23 UmL^{-1}, respectively. In the control fish, maximum activity was observed in the liver, while minimum activity at 1/3rd was measured in muscles. Overall trend of SOD activity in organs was liver>gills>kidney>muscles. SOD activity in *Cirrhina mrigala* increased in all treatments with increasing exposure duration as, at day-5 39.84±6.75 UmL^{-1} activity was observed which increased at 10 and 15-days with mean values of 44.44±4.14 UmL^{-1} and 45.30±10.41 UmL^{-1}, respectively while at 30-day exposure period, SOD activity started decreasing to 36.69±7.59 UmL^{-1}. Comparisons of means reveals maximum activity of SOD was observed in 1/4th of LC_{50} exposed *Cirrhina mrigala* with the mean value of 44.00±4.64 UmL^{-1} while significantly lower SOD activity was measured in 1/3rd of Fe+Mn mixture exposed fish.

Figure 1 shows the exposure dose and time dependent comparison of SOD activity in two fish species viz. *C. catla* and *C. mrigala*, exposed to sub-lethal concentrations of Fe+Mn mixture for different exposure periods. The body organs of metals treated *Cirrhina mrigala* exhibited statistically significant increased SOD activity as compared to *Catla catla*. Same trend was observed in case of treatments viz., 1/4th and 1/3rd concentrations of LC_{50} of Fe+Mn mixture for different durations.

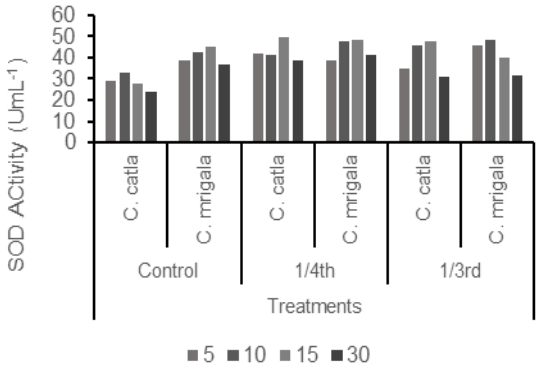

Figure 1. Comparison of exposure dose and time dependent SOD activity in two fish species

Physico-chemical parameters

Physico-chemical parameters viz. temperature, pH, total hardness, total ammonia, DO, CO_2, Ca and Mg were recorded during the experimental trials and presented in table 3 for *Catla catla* experimental media and in table 4 for *Cirrhina mrigala* media.

Table 2. Superoxide dismutase activity (UmL^{-1}) in the selected organs of *Cirrhina mrigala*, after chronic exposure to Fe+Mn mixture

Treatment	Duration	Organs				Overall Means
		Liver	Kidney	Gills	Muscles	
Control	05	45.09±2.79	40.33±2.52	38.82±2.65	31.38±2.55	38.91±5.68c
	10	47.29±2.87	42.16±2.68	46.94±2.71	34.95±1.69	42.84±5.75b
	15	52.27±3.11	45.31±2.73	43.38±2.70	40.15±2.50	45.28±5.12a
	30	43.16±2.55	35.69±1.85	38.11±1.77	30.31±1.48	36.82±5.34d
	Overall Means	46.95±3.93a	40.87±4.02c	41.81±4.13b	34.20±4.44d	--
1/4th	05	43.10±1.86	38.85±1.59	48.61±2.50	24.56±0.77	38.78±10.29d
	10	52.88±2.76	44.11±2.62	43.11±2.23	49.58±3.13	47.42±4.62b
	15	61.04±3.55	48.96±2.79	57.08±5.50	26.39±0.85	48.37±15.49a
	30	52.17±2.31	39.51±1.83	47.46±2.12	26.54±1.65	41.42±11.21c
	Overall Means	52.30±7.33a	42.86±4.69c	49.06±5.84b	31.77±11.91d	--
1/3rd	05	48.11±2.66	46.31±2.48	38.84±1.76	34.09±1.39	41.84±6.54c
	10	51.78±3.30	40.85±1.99	36.49±1.92	43.11±2.25	43.06±6.43a
	15	55.75±3.46	37.50±1.41	47.31±2.87	28.47±0.93	42.26±11.84b
	30	38.54±1.79	27.80±0.98	36.10±1.80	24.83±0.88	31.82±6.54d
	Overall Means	48.54±7.36a	38.12±7.78c	39.68±5.22b	32.62±7.96d	--

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Table 3. Mean values of physico-chemical variables determined during Fe+Mn mixture exposure to *Catla catla*

Treatment	Duration (days)	Temp. (°C)	pH	Total Hardness (mgL^{-1})	Total Ammonia (mgL^{-1})	Dissolved Oxygen (mgL^{-1})	Carbon Dioxide (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})
Control	5	27.98±0.08	7.51±0.08	224.98±0.07	0.21±0.05	5.79±0.31	1.38±0.27	21.16±2.16	43.02±3.21
	10	28.00±0.09	7.53±0.10	225.00±0.12	0.36±0.07	5.72±0.30	1.40±0.28	21.20±2.18	43.00±3.20
	15	28.01±0.12	7.50±0.07	225.03±0.19	0.50±0.09	5.66±0.28	1.43±0.26	21.23±2.19	42.98±3.18
	30	28.03±0.14	7.52±0.09	225.02±0.17	0.71±0.11	5.60±0.26	1.50±0.25	21.30±2.22	42.94±3.16
	Mean±SD	28.00±0.10	7.51±0.08	225.01±0.11	0.44±0.08	5.69±0.28	1.43±0.26	21.22±2.18	42.98±3.18
$1/4^{th}$	5	28.00±0.09	7.50±0.08	225.29±0.14	0.76±0.13	5.80±0.31	1.47±0.26	23.36±2.23	41.65±3.16
	10	28.01±0.12	7.55±0.10	225.35±0.16	0.88±0.15	5.66±0.29	1.50±0.28	23.66±2.25	41.46±3.15
	15	28.02±0.13	7.53±0.11	225.44±0.20	1.27±0.21	5.61±0.25	1.59±0.27	23.98±2.29	41.26±3.12
	30	28.01±0.11	7.49±0.07	225.50±0.27	1.03±0.17	5.56±0.20	1.53±0.26	24.61±2.32	40.87±3.09
	Mean±SD	28.01±0.11	7.52±0.09	225.39±0.19	0.98±0.16	5.66±0.26	1.52±0.25	23.90±2.27	41.56±3.13
$1/3^{rd}$	5	28.00±0.11	7.49±0.06	225.42±0.17	0.70±0.10	5.38±0.18	1.68±0.22	24.88±2.26	40.70±3.10
	10	28.02±0.13	7.53±0.07	225.58±0.15	1.21±0.19	5.34±0.15	1.59±0.25	25.59±2.33	40.26±3.04
	15	28.01±0.12	7.55±0.08	225.77±0.20	0.97±0.14	5.23±0.13	1.75±0.27	26.01±2.36	39.99±2.98
	30	28.00±0.10	7.59±0.09	225.83±0.26	1.43±0.24	5.19±0.14	1.84±0.27	26.58±2.39	39.64±2.89
	Mean±SD	28.00±0.11	7.54±0.07	225.65±0.19	1.07±0.16	5.28±0.15	1.72±0.28	25.76±2.33	40.15±3.00

Table 4. Mean values of physico-chemical variables determined during Fe+Mn mixture exposure to *Cirrhina mrigala*

Treatment	Duration (days)	Temp. (°C)	pH	Total hardness (mgL^{-1})	Total Ammonia (mgL^{-1})	Dissolved oxygen (mgL^{-1})	Carbon dioxide (mgL^{-1})	Calcium (mgL^{-1})	Magnesium (mgL^{-1})
Control	5	28.00±0.07	7.53±0.09	224.97±0.07	0.19±0.06	5.82±0.32	1.40±0.28	21.21±2.17	43.11±3.23
	10	28.01±0.10	7.54±0.11	225.03±0.12	0.38±0.08	5.74±0.31	1.44±0.29	21.24±2.19	43.14±3.21
	15	28.03±0.13	7.52±0.08	225.07±0.19	0.53±0.10	5.67±0.29	1.46±0.27	21.29±2.20	43.15±3.19
	30	28.00±0.10	7.51±0.10	225.04±0.17	0.73±0.12	5.62±0.27	1.50±0.26	21.33±2.23	43.14±3.17
	Mean±SD	28.01±0.10	7.52±0.09	225.03±0.11	0.46±0.09	5.71±0.29	1.45±0.27	21.27±2.19	43.13±3.20
$1/4^{th}$	5	28.01±0.08	7.51±0.09	225.32±0.14	0.77±0.14	5.78±0.32	1.48±0.27	23.49±2.25	41.72±3.18
	10	28.03±0.12	7.57±0.11	225.41±0.16	0.91±0.16	5.68±0.30	1.51±0.29	23.62±2.27	41.66±3.16
	15	28.04±0.14	7.56±0.12	225.55±0.20	1.26±0.22	5.64±0.26	1.57±0.28	24.22±2.30	41.30±3.14
	30	28.02±0.11	7.47±0.08	225.61±0.27	1.01±0.18	5.61±0.21	1.62±0.27	24.69±2.33	41.04±3.10
	Mean±SD	28.02±0.11	7.53±0.10	225.47±0.19	0.99±0.17	5.68±0.27	1.54±0.26	24.00±2.29	41.43±3.14
$1/3^{rd}$	5	28.00±0.08	7.46±0.07	225.45±0.17	0.71±0.11	5.34±0.19	1.59±0.23	24.59±2.25	41.07±3.12
	10	28.03±0.11	7.57±0.08	225.62±0.15	1.02±0.15	5.31±0.16	1.66±0.26	25.81±2.34	40.29±3.05
	15	28.02±0.12	7.56±0.09	225.79±0.20	1.22±0.20	5.25±0.14	1.88±0.28	26.03±2.38	39.98±2.99
	30	28.04±0.14	7.61±0.10	225.88±0.26	1.43±0.25	5.28±0.15	1.78±0.30	26.57±2.41	39.89±2.91
	Mean±SD	28.02±0.11	7.55±0.08	225.68±0.19	1.10±0.17	5.30±0.16	1.73±0.27	25.75±2.34	40.31±3.02

DISCUSSION

Heavy metals are among the toxic aquatic contaminants and their excessive and continuous addition to aquatic ecosystems has raised environmental and health concerns globally (Vutukuru, 2003). As metals discharged from diversified sources in the aquatic environment so they are present in the mixture form, however most eco-toxicological studies centered on the effects of individual metals (Lange *et al*., 2002). In the form of mixtures, metals interaction even at lower concentration effects the growth as well as biochemical processes of different fish species (Birceanu *et al*., 2008). Iron and manganese are naturally occurring micronutrients in aquatic ecosystems. Both these metals work as a cofactor in many enzymatic reactions as well as structural units of many biochemical activities (Holmes, 2010). Like all others aquatic aerobic organisms, fish have an antioxidant defense system to neutralize the effect of reactive oxygen species (ROS). Superoxide dismutase (SOD) is one of the antioxidant enzyme that responds against oxidative stress. SOD catalyzes the conversion of superoxide radicals to O_2 and H_2O_2 and in this way protect the organism from superoxide-induced oxidative stress (Vutukuru *et al*., 2006).

After exposure to sub-lethal concentrations ($1/4^{th}$ and $1/3^{rd}$) of Fe+Mn mixture for 30 days, SOD activity was found to be increased as compared to control in both fish species, *Catla catla* and *Cirrhina mrigala*. Rajasekar and Venkatakrishnaiah (2016) also reported significantly (P<0.05) increased SOD activity in *Catla catla* exposed to three sub-lethal concentrations of copper. During the present research work, it was observed that the gills and liver of *Catla catla* showed maximum activity of SOD while in *Cirrhina mrigala*, liver exhibits significant increase in the activity of SOD as compared to gills, kidney and muscle as liver

enzymes are more sensitive to stress caused by metals. Dalzell and Macfarlane (2000) suggested that possible mechanism of Fe toxicity in gills is by clogging which disrupts the respiratory process. Liver is the site of free radical generation and multiple oxidative reactions (Gul *et al*., 2004). Omar *et al*. (2014) reported significantly higher accumulation of iron in liver followed by kidney and gills. It was also observed that with decreased in the concentration of Fe+Mn mixture there was significant increase in the SOD activity as compared to control in both fish species. These results are in line with the findings of Liu (2006) who reported that with increasing concentration of Cu from 0.0025-0.25 mgL^{-1}, the activity of SOD decreased up to 0.05 but significantly increased at 0.25 mgL^{-1} Cu concentration. In the 30 days experiment, it was noted that SOD activity significantly increased up to 15 days of metals mixture (MM) exposure then it started decreasing to almost basal level after 30-day of MM exposure. This decrease in the activity of SOD might be due to the reactions of amino acid residues with the products of lipid peroxidation that can severely affects protein function causing decreased in the activity of enzyme (Bagnyukova *et al*., 2006) or due to increase in the production of ROS by Fenton reactions (Ates *et al*., 2008). In comparison to *Catla catla*, *Cirrhina mrigala* showed greater activity of SOD after 15 days of Fe+Mn mixture exposure.

Results of this research are in conformity with the findings of Trenzado *et al*. (2006) who reported the significant increase in superoxide dismutase (SOD) activity (834.63±28.15 and 967.63±48.69 UmL^{-1}) in the liver of both trout and sturgeon. Sturgeon showed maximum SOD activity because it had higher fat content in muscles and liver as compared to trout, which protects the fish against oxidative stress. Farombi *et al*. (2007) also noted that in the liver of *Clarias gariepinus* the SOD activity increased by 61% compared to control when exposed to heavy metals. Jastrzebska (2010) conducted an experiment to determine the relation between concentration of metals and response of antioxidant enzymes. Results of experiment showed that with the increase in concentration of cadmium (Cd) and lead (Pb) the activity of SOD decreased significantly. Kong *et al*. (2013) observed that at 0.1 mgL^{-1} of copper exposure, SOD activity significantly increased in *Carassius auratus* as it is the first line of defense but at increased concentrations i.e. 0.4 mgL^{-1} and 0.7 mgL^{-1}, copper significantly inhibited the activity of SOD resulted in the oxidative stress. Similarly results of Khalid *et al*. (2015) are also in conformity with the findings of present research work. They observed that with the increase in heavy metals concentration, the level of SOD decreased in *Labeo rohita*.

Saliu and Kafilat (2012) reported significant increase in the activity of SOD in liver but after 28 days of Pb (NO_3) exposure, the SOD activity (1.37±0.27 UmL^{-1}) was lower than control group (2.37±0.62 UmL^{-1}). Significant elevation in the SOD activity in the liver was observed by Basha and Rani (2003). They noted the increase in the SOD activity in Tilapia from day 7 to day 15 and then the activity decreased after 30-day of metal exposure. They suggested that this increase in the enzyme production might be a defense of an organism against metal toxicity before metallothioneins induction. Eyckmans *et al*. (2011) studied that rainbow trout responded to copper toxicity with an increased in the SOD activity but after a month of metal exposure all antioxidant enzymes including SOD returned to basal levels. They also observed the interspecies differences between rainbow trout, gibel carp and common carp.

CONCLUSION

The mixture of two heavy metals Fe+Mn caused a significant oxidative stress in the tissues of *Catla catla* and *Cirrhina mrigala*. Among the two major carps, C. catla showed more stress as the SOD activity was significantly higher as compared to C. mrigala when exposed to various doses of Fe+Mn for different durations.

AUTHOR'S CONTRIBUTION

H. Tayyab: Executed the Research work and wrote the manuscript

F. Latif: Supervised the research and wrote the manuscript

S. Aziz: Helped in statistical analysis of data

R. Zubair: Helped in research project execution and manuscript write-up

J. Aslam: Helped in research project execution and manuscript write-up

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