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ANTIOXIDANT ENZYMES ACTIVITY DURING ACUTE TOXICITY OF CHROMIUM AND CADMIUM TO CHANNA MARULIUS AND WALLAGO ATTU

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Acute toxicity tests (96-hr LC_{50} and lethal concentration) of chromium (Cr) and cadmium (Cd) were conducted with two fish species viz. *Channa marulius* and *Wallago attu.* At the end of each trial, the fish were dissected and their organs viz. gills, kidney and liver were isolated for the determination of antioxidant enzymes activity. Cadmium was significantly more toxic than chromium with the mean 96-hr LC_{50} values of 75.70 ± 0.91 and 94.86 ± 0.96 mg L^{-1}, respectively. *C. marulius* was significantly less sensitive than *W. attu.* Activity of superoxide dismutase increased with increasing metallic ion concentrations in the test mediums for both fish species. However, the activity of catalase and peroxidase decreased by increasing the concentration of metallic ions in the test mediums. The fish kept in control conditions (without metal stress) showed maximum activity of catalase and peroxidase. The fish, *C. marulius* accumulates significantly higher contents of chromium than cadmium. Among fish organs, liver appeared as a target organ that accumulate significantly higher contents of chromium followed by that of kidney, gills, fins, skin, muscle and bones. The *W. attu* followed almost the similar trend as that of *C. marulius* for the accumulation of metals. The accumulation order of metals in fish organs were liver>kidney>gills>fins>skin>muscle>bones for both fish species.
Keywords: Acute toxicity, heavy metals, antioxidant enzymes, bioaccumulation, fish

INTRODUCTION

Heavy metals pollution in aquatic ecosystem is global issue due to persistence and continuous accumulation of these pollutants in aquatic environment (Hyun *et al.,* 2006). The biological degradation of heavy metals is not possible because these metals store in the organs system of aquatic organisms and become deleterious and consequently pass to other living organism like humans who consume these aquatic individuals as food (Ashraf, 2005). In ecotoxicology, heavy metals have gained significant consideration because of their severe toxicity and amassing tendency in the aquatic biota (Javed, 2004).

In freshwater ecosystem, fish is the most indicative factor to maintain pollution in aquatic ecosystem (Rashed, 2001). Fish due to high level in the food web may concentrate some metals and their residues from the water several times greater than in the ambient water. Accumulation of metals in organs of fish varies from organ to organ due to their affinity. All this process showed different metals concentrations in organs system at different levels (Bervoets *et al*., 2001). The effluents discharged from electroplating industry, leather tanneries, photographic, dying and pharmaceutical have chromium in different valence state (Vutukuru, 2005). Chromium once enters in the ecosystem than due to its half life; it remains in that system and valence state is changed. Mainly trivalent (Cr^{3+}) and hexavalent states (Cr^{6+}) are found in waters. Trivalent state is less toxic than that of hexavalent. Some salts that are used as supplementary in the diet may contain chromium (Cr^{3+}) in trace amount (Lushchak *et al.,* 2009). Cadmium is a toxic and non-essential element that is a widespread aquatic environmental pollutant. It is specifically found in coastal areas and estuaries where it is predominantly released by human activities such as smelting, mining, metal-plating industries and human products such as plastics, ceramics, glass, vehicle tires, steel, alloys, paints and batteries (Thompson and Bannigan, 2008). To measure the susceptibility and survival potential of aquatic organisms for particular toxic pollutant 96-hr LC_{50} and lethal tests are used. The LC_{50} values measures toxicity of pollutants (Eaton *et al.,* 1995). Omnivorous fish may bio-accumulate and concentrate more heavy metals than the carnivorous fish in natural habitats (Yousafzai *et al.,* 2010).

Riverine system of Pakistan is polluted and these pollutants are adversely affecting the indigenous fish fauna of Pakistan. The *C. marulius* and *W. attu* are threatened in Pakistan due to heavy load of pollutants such as heavy metals. The tolerance limits of these fish species against chromium and cadmium have not been determined. Therefore, this research work was planned to determine the acute (LC_{50} and lethal) response of *C. marulius* and *W. attu* during 96-hr LC_{50} and lethal exposure.

MATERIALS AND METHODS

***Fish fingerlings*:** The fingerlings of *C. marulius* and *W. attu* were collected from their natural breeding grounds (Head Chanawa and Head Khanki). The fingerlings of both fish species were transported to the laboratory and placed in cemented tanks having 1000 liters water capacity. The acclimation period in the laboratory was lasted for 15 days having photoperiod of 12h Light: 12h Dark. The fingerlings were fed with diet, containing 40% crude protein.

***Chemicals*:** The pure chloride compound of cadmium and nitrate compound of chromium were used as metal toxicant. Desired concentrations of Cr and Cd (Merk) were prepared by dissolving an appropriate volume of stock solution in tap water (APHA, 1998).

***Metals acute toxicity assays*:** The 96-hr LC_{50} and lethal concentrations were determined in static bioassay system. Metal's toxicity concentration for each fish species were started from 0 and increased as 0.05 and 5 mg L^{-1} (as total concentration) for low and high metals concentrations, respectively. The fish were not fed during acute toxicity trials. In each aquarium the concentration of metal was increased gradually in order to avoid the fish from stress. Continuous air was supplied to all the test and control mediums with an air pump through capillary network. Fish mortality data obtained against each concentration of metals during 96 h test duration was recorded. The acute toxicity tests were performed at constant water temperature (28°C), pH (7.50) and total hardness (150 mg L^{-1}) in static bioassay systems. The acute toxicity bioassay procedure, based on standard method (A.P.H.A. 1998) was conducted to determine 96-h LC_{50} and lethal concentrations of Cr and Cd for each fish species.

***Enzyme assays*:** The fish used in the acute (96-hr LC_{50}) and lethal test trails were weighed and removed from the media. All the fish were dissected and organs viz. liver, kidney and gills were removed. These organs were kept at -80°C for the further enzyme assays and biochemical analyses.

***Preparation of extract*:** To remove RBCs the dissected organs of each fish were washed with phosphate buffer (pH 6.5), Organs were weighed and homogenate was prepared in phosphate buffer (0.2M, pH 6.5) with a ratio of 1: 4, respectively. These tissues homogenates were then centrifuged at 10,000 rpm for 15 minutes at 4°C. The clear supernatant was preserved and used for further enzyme analysis.

***1. Superoxide dismutase assay*:** The activity of superoxide dismutase was determined by measuring its ability to inhibit the O^{2-} dependent reaction or to inhibit the photo-reduction of nitro-blue tetrazolium (NBT) by superoxide (Giannopolitis and Ries, 1977).

***2. Catalase assay*:** The crude enzyme was subjected to enzyme assay and the activities of catalase were measured by following the method of Chance and Maehly (1995) with some modifications. Catalase activity was concluded by measuring its ability to decrease the H_2O_2 concentration per minute at 240 nm.

***3. Peroxidase*:** For the determination of peroxidase activity, crude enzyme was subjected to enzyme assay according to Civello *et al*. (1995). Activity of peroxides was determined by measuring its ability to decrease the concentration of H_2O_2 at 470 nm.

***Tissue distribution assays*:** The fish were removed from the experiment and after being lightly blotted. Wet weight and length were measured and recorded. The fish were dissected and their body organs viz. gills, liver, kidney, fins, bones, muscle and skin were isolated for the determination of their respective exposure metals concentrations through Atomic Absorption Spectrophotometer (Analyst-400).

***Statistical analyses of data*:** Three replicates were used for the whole experiment and data obtained during acute toxicity phase on fish morality (%) were analyzed by using Probit Analysis Method (Finney, 1971). The data on enzymes studies and metals bio-accumulation patterns of test mediums were subjected to statistical analyses by using STATISTICA, MSTATC, MICROSTAT by following Steel *et al.* (1997). Analysis of variance and comparison of means were performed by using Tukey's/Student Newnan-Keul tests to find-out statistical differences among different variables under study.

RESULTS AND DISCUSSION

The experimental fish viz. *C. marulius* and *W. attu,* separately, were tested to determine their 96-hr LC_{50} and lethal concentrations for chromium and cadmium. Both the fish species were exposed to different concentrations of metals Figures 1 and 2.

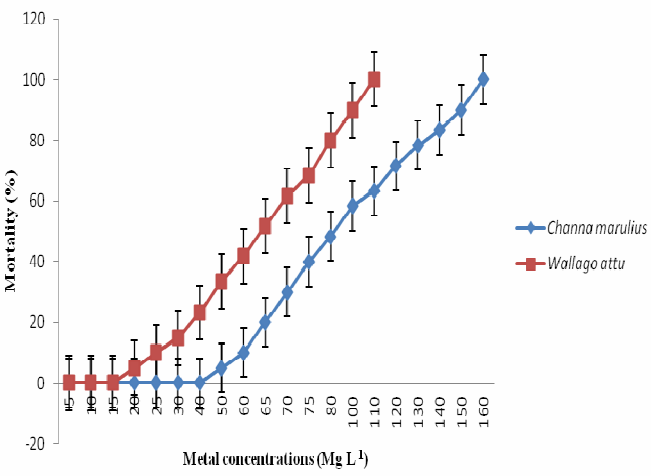

Figure 1. The percentage mortality of *C. marulius* and *W. attu* at different chromium concentrations during 96-hr acute toxicity tests.

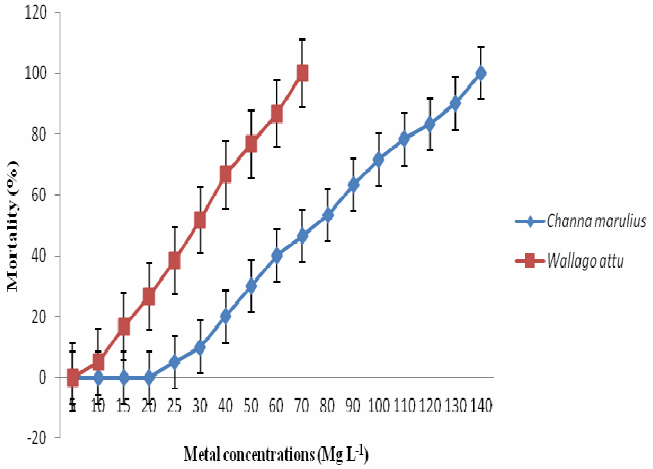

Figure 2. The percentage mortality of *C. marulius* and *W. attu* at different cadmium concentrations during 96-hr acute toxicity tests.

***96-HR LC_{50} and lethal concentrations*:** Statistical analysis revealed that both the fish species showed significant differences towards metals sensitivity. Cadmium was significantly more toxic than chromium with the mean 96-hr LC_{50} values of 54.32 ± 21.37 and 78.11 ± 16.73 mgL^{-1}. The overall mean 96-hr LC_{50} values calculated for *C. marulius* and *W. attu* showed that *C. marulius* was significantly less sensitive than *W. attu.* The overall response of both fish species, for their tolerance against metals varied significantly at $p<0.01$. The exposure of metals to *C. marulius* showed least sensitivity while *W. attu* showed significantly highest sensitivity in terms of lethal exposure. The sensitivity of metals for *C. marulius* and *W. attu* was cadmium>chromium with the mean lethal values of, 121.78 ± 44.24 and 153.77 ± 30.42 mgL^{-1}, respectively (Table 1). The 96-hr LC_{50} values measurement is a very important factor to check the susceptibility of fish to pollutions. The sensitivity of fish varies from species to species and metals to metals. The metal that is toxic at low level concentration to one individual may be non-toxic or less toxic to other animal at similar or higher concentration (Shah and Altindu, 2005). LC_{50} values for the same toxicant differ from individual to individual due to the mode of action and responses of the animals (King, 1992). Sanjay *et al*. (2006) exposed *C. marulius* to Cr (VI) for 96 hours. They observed that toxicity of Cr to fish is dose and time dependent *i.e.,* with the increase in metal concentration and time, the mortality was also increased. During present research work this trend was also noted that toxicity of Cr (VI) increased with concentration. Tiwari *et al.* (2011) studied the toxicity of cadmium for freshwater teleost, *Channa punctata* (Bloch) at 24, 48, 72 and 96-hr exposure durations. The LC_{50} values with 95% confidence level were estimated by SPSS as 26.88 (21.69-71.68), 18.76 (17.13-20.81), 16.70 (14.77-17.96) and 14.95 (13.13-15.88) mgL^{-1} for dissolved metal concentrations, respectively.

***Antioxidant enzyme activity*:** During acute exposure the activity of antioxidant enzymes viz. superoxide dismutase, catalase and peroxidase were also studied in the test and control mediums. The activities of these enzymes were determined in fish organs viz. kidney, gills and liver.

***Chromium*:** Overall highest mean activity of superoxide dismutase in *C. marulius* and *W. attu* liver was found as 100.69 and 96.97 U/ml followed by that of kidney (91.23 and 91.76 U/ml) and gills (88.80 and 82.48 U/ml). The data showed increased superoxide dismutase activity with increasing metallic ion concentrations in the test mediums (Fig. 3). However, the control (unstressed) fish showed least activity of superoxide dismutase. Superoxide dismutase activity in organs of both fish species was statistically different. The Overall mean activity of catalase and peroxidase in the liver, kidney and gills of *C. marulius* were noted as 313.002 and 0.348 U/ml; 280.451 and 0.200 U/ml; 239.256 and 0.196 U/ml, respectively. The activity of catalase and peroxidase in *W. attu* organs followed almost the similar trend as that of *C. marulius*. However, the

Table 1. Responses of both fish species for their 96-hr LC_{50} and lethal concentrations (mgL^{-1}) of chromium and cadmium.

Treatments	Metals	Fish Species		
		C. marulius	*W. attu*	*Overall Means
96-hr LC_{50}	Chromium	94.85±1.35 a	61.38±1.67 b	78.11±16.73 A
	Cadmium	75.70±1.29 a	32.95±0.51 b	54.32±21.37 B
	Means	85.27±9.57 A	47.16±14.21 B	
96-hr lethal concentration	Chromium	184.19±3.59 a	123.35±1.19 b	153.77±30.42 A
	Cadmium	166.03±3.58 a	77.54±2.91 b	121.78±44.24 B
	Means	175.11±9.08 A	100.44±22.90 B	

Means with the same letters (small) in a single row and the *overall means in a single column (capital) are statistically similar at $P<0.05$.

peroxidase activity in *W. attu* was maximum in kidney followed by that of liver and gills (Figure 3). Control fish showed maximum activity of catalase and peroxidase. The activity of catalase and peroxidase decreased by increasing metal concentration in the test medium that indicate stressed conditions. The difference between catalase and peroxidase activity in both fish species were statistically significant at $p<0.01$. Velma and Tchounwou (2010) were reported increased activity in superoxide dismutase at all concentrations of chromium as compared to control fish, *Carassius auratus*. They also reported significant decrease in catalase activity with increasing chromium concentration in the test medium while control fish showed normal activity of catalase. Kubrak *et al*. (2010) reported depressed activity of catalase in kidney, gills and liver of fish exposed to (Cr^{+3}) for short period. Decrease in the activity of catalase in chromium exposed fish had also been reported by Vasylkiv *et al*. (2010).

***Cadmium*:** The highest mean activity of superoxide dismutase was observed in *C. marulius* liver (258.278 U/ml) followed by that of kidney (245.571 U/ml) and gills (198.029 U/ml). However, *W. attu* kidney showed minimum activity of superoxide dismutase as 84.201 U/ml. Statistical analysis showed statistically significant difference between

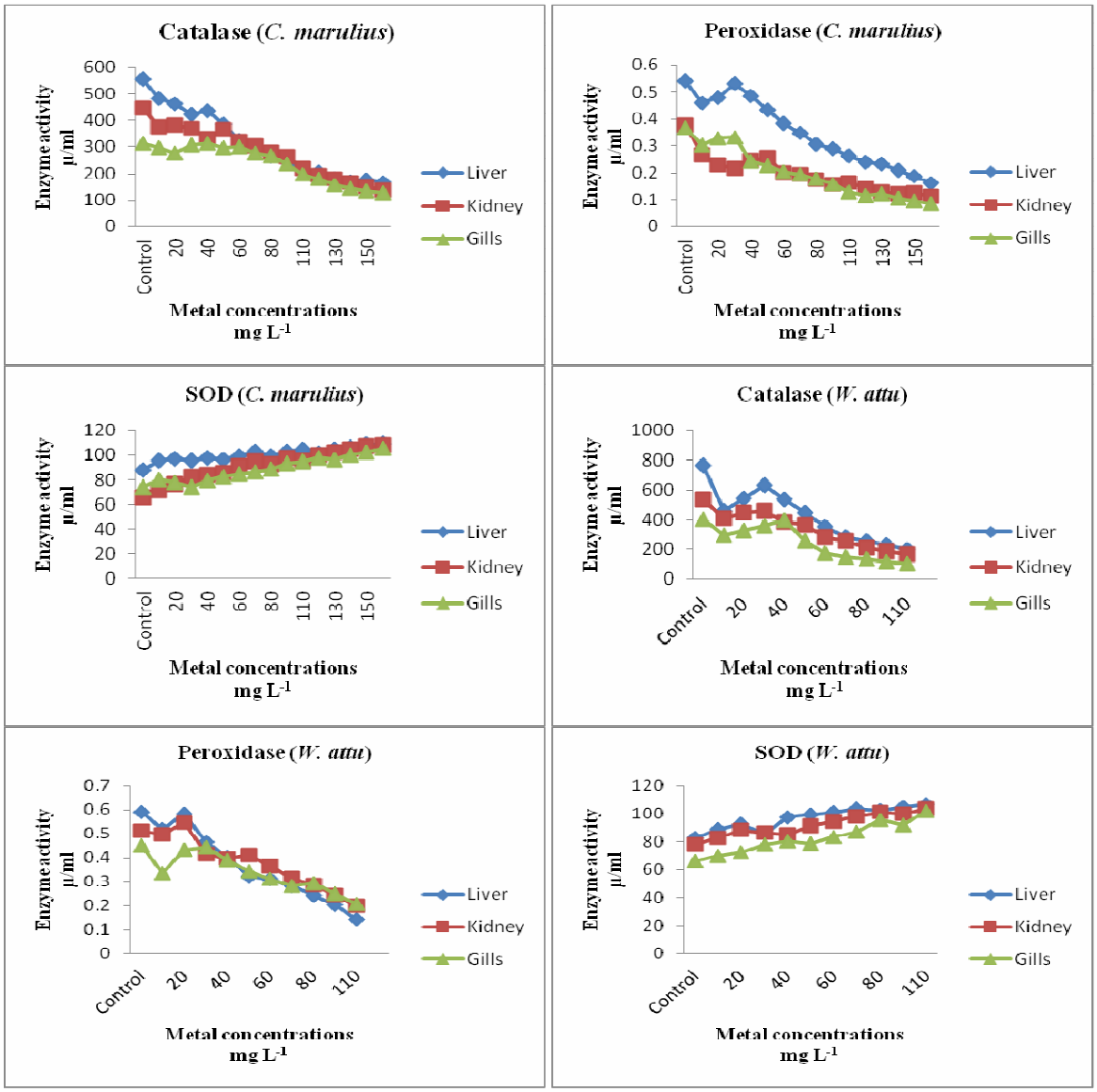

Figure 3. Antioxidant enzymes activity of chromium stressed *C. marulius* and *W. attu*.

C. marulius and *W. attu*. Catalase and peroxidase activity in *C. marulius* liver, kidney and gills were noted as 530.201 and 0.382; 397.266 and 0.198; 299.551 and 0.192 U/ml, respectively. Maximum activity of catalase and peroxidase were noted in control (unstressed) than that of metal exposed fish. The activity levels of catalase and peroxidase in liver, kidney and gills of *C. marulius* and *W. attu* showed statistically significant ($p<0.01$) differences Figure 4. Cadmium accumulation in fish organs also affects the activity of SOD in catfish (*Clarias gariepinus*). An increase in SOD activity in fish with increasing metal concentration was noted by (Asagba *et al*., 2008; Vieria *et al.,* 2009). Ahmad *et al.* (2006) reported that organ specificity depends on bioaccumulation of metals and defensive mechanism of the particular organ. Peroxidase activity in fish liver decreased when compared with control. Vieria *et al*. (2009) also reported an increase in antioxidant enzyme activity in eustrine fish exposed to copper and mercury for 96-hr.

***Metals accumulation in fish organs*: 96-hr LC_{50}:** The fish, *C. marulius* accumulate significantly higher chromium than cadmium. Among fish organs liver accumulate significantly higher chromium concentration of 248.33±4.42 μgg^{-1} followed by that of kidney (219.44±0.80 μgg^{-1}), gills (142.66±1.95 μgg^{-1}), fins (70.66±1.51 μgg^{-1}), skin (43.66±0.16 μgg^{-1}), muscle (8.49±0.21 μgg^{-1}) and bones (5.87±0.06 μgg^{-1}) Table 2. The *W. attu* followed almost the

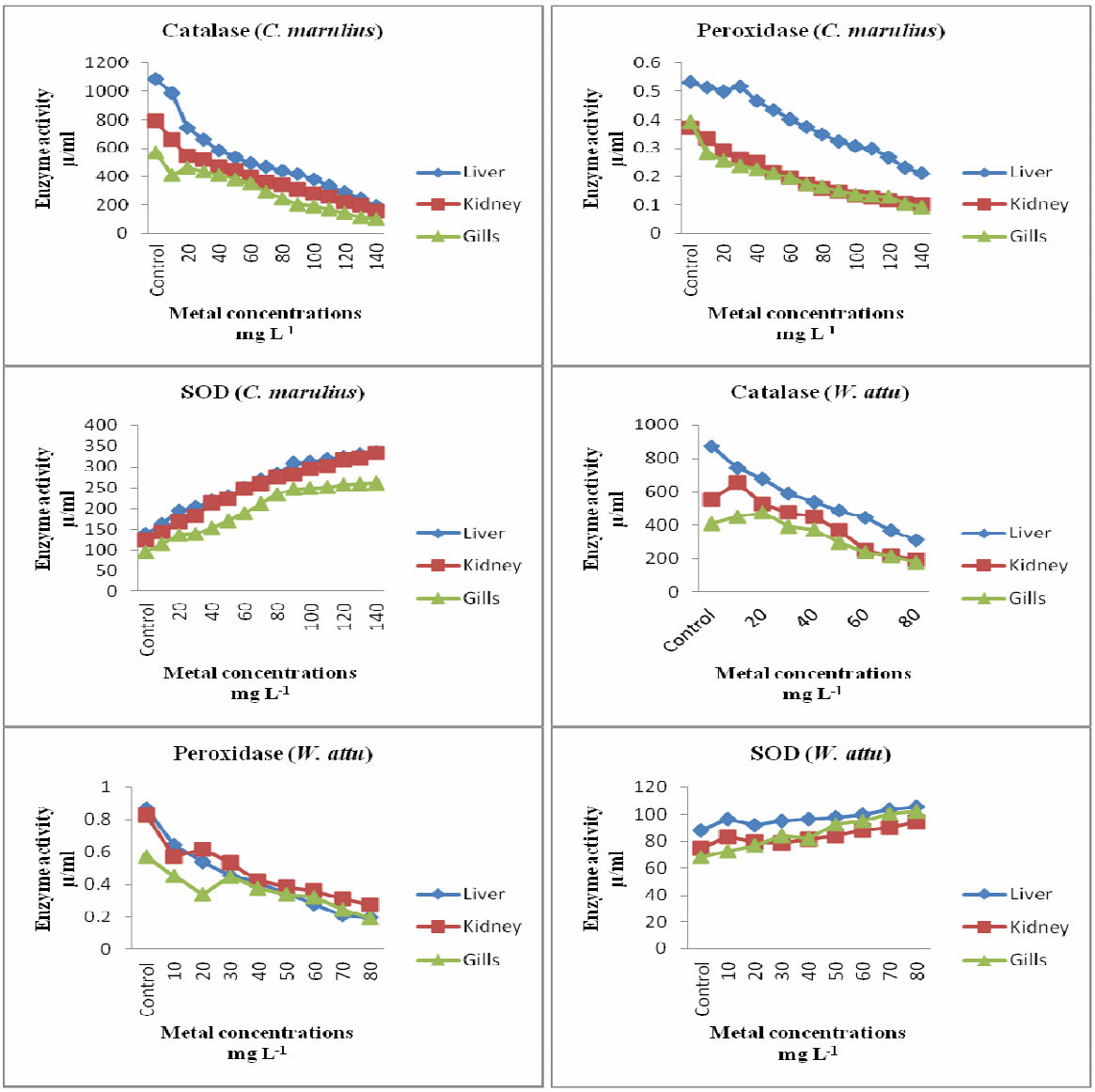

Figure 4. Antioxidant enzymes activity of cadmium stressed *C. marulius* and *W. attu*.

Table 2. Accumulation of chromium and cadmium (µg g^{-1}) in fish body organs during 96-hr acute toxicity exposures.

Metals	Species	Organs							*Metals x Species Overall Mean±SE
		Gills	Liver	Kidney	Fins	Bones	Muscles	Skin	
i. At 96-hr LC_{50}									
Chromium	*C. marulius*	142.66±1.95c	248.33±4.42a	219.44±0.80b	70.66±1.51d	5.87±0.06g	8.49±0.21f	43.66±0.16e	105.59±20.56A
	W. attu	113.89±0.42c	205.22±2.25a	200.57±1.02b	58.66±0.05d	2.59±0.01g	7.09±0.06f	29.21±0.03e	88.18±17.98B
	Means	128.27±14.38	226.77±21.55	210.00±9.43	64.66±6.00	4.23±1.64	7.79±0.70	36.43±7.22	
	C. marulius	114.33±1.20d	169.70±0.94b	190.54±2.04a	125.00±1.69c	9.53±0.06g	12.38±0.07f	37.13±0.21e	94.09±15.46A
Cadmium	*W. attu*	105.90±1.63c	136.14±0.68a	130.39±1.85b	95.44±0.39d	6.25±0.06g	9.52±0.08f	28.33±0.20e	73.14±11.76B
	Means	110.11±4.21	152.92±16.78	160.46±30.07	110.22±14.78	7.89±1.64	10.95±1.43	32.73±4.40	
ii. At 96-hr lethal concentration									
Chromium	*C. marulius*	176.49±4.57c	350.01±2.49b	371.66±5.49a	84.40±2.58d	8.57±0.23g	16.69±0.15f	65.62±1.00e	153.35±31.52A
	W. attu	136.32±2.12c	279.50±11.10a	264.89±4.34b	73.14±2.01d	5.36±0.07g	12.69±0.28f	47.99±0.37e	117.13±23.75B
	Means	156.40±20.08	314.75±35.25	318.27±53.38	78.77±5.63	6.96±1.60	14.69±2.00	56.80±8.81	
Cadmium	*C. marulius*	159.31±3.52c	242.77±2.83a	218.37±3.00b	96.70±1.14d	7.79±0.06g	13.36±0.05f	56.03±0.38e	113.48±19.76A
	W. attu	131.19±1.28c	209.52±3.33a	193.97±1.22b	82.98±0.71d	3.85±0.03g	9.01±0.09f	41.29±0.43e	95.97±17.52B
	Means	145.25±14.06	226.14±16.62	206.17±12.20	89.84±6.86	5.82±1.97	11.18±2.17	48.66±7.37	

Means with same letters in a single row for each metal among organs and *column between species are statistically similar at $p< 0.05$.

similar trend as that of *C. marulius* for the accumulation of metals. The order of cadmium accumulation in fish organs were liver>kidney>gills>fins>skin>muscle>bones in both fish species. The differences among fish species for their ability to accumulate different metals in bodies appeared species specific (Abdullah *et al.,* 2011). The fish organs showed higher variations to concentrate Cr in their bodies during acute exposures. However, liver and kidney exhibited significantly higher tendencies to accumulate Cr (Azmat and Javed, 2011). Yousafzai *et al.* (2012) reported significantly less amount of metals in the muscle of *Cyprinus carpio* while fish liver accumulated significantly higher concentration of metals. Shukla *et al.* (2007) reported that concentration of zinc, cadmium and copper was maximum in liver and minimum in muscle of *Channa punctatus*. The metals accumulation varies among organs.

***96-hr lethal*:** Kidney of *C. marulius* was the organ that accumulates higher chromium concentration of 371.66±5.49 µgg^{-1} than liver that accumulated this metal as 350.01±2.49 µgg^{-1}, Table 2. The accumulation of metals in different organs of *W. attu* showed the order i.e. liver>kidney> gills>fins>skin>muscles>bones. Metals comparison in both fish species showed that chromium accumulated maximum than that of cadmium. Vinodhini and Narayanan (2008) observed the metals accumulation sequence in fish liver and gills as Pb>Cd>Ni>Cr and Cd>Pb>Ni>Cr, respectively. Ashraf *et al.* (2012) found significantly higher levels of Sn>Pb>Zn>Cu>As in the body of *Rasbora elgans,* followed by that of *Trichogaster trichopterus* and *Oxyeleotris marmorata*. The liver plays an important role in accumulation and detoxification of heavy metals and the sequence of metals accumulation in the liver of *Wallago attu* was Pb>Cr>Zn>Cu>Ni>Cd (Yousafzai, 2010).

***Conclusion*:** The metals (Chromium and Cadmium) 96-hr LC_{50} and lethal responses by both, *C. marulius* and *W. attu* showed statistically significant differences. Cadmium was significantly more toxic than chromium. Between the two fish species *W. attu* was found significantly more sensitive in terms of 96-hr LC_{50} and lethal responses.

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