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BIOCHEMICAL CHANGES INDUCED IN *TILAPIA MOSSAMBICUS* DURING COPPER AND CADMIUM ACUTE INTOXICATION

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Keywords:

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ABSTRACT

Copper and cadmium is a trace element which is essential to the function of specific proteins and enzymes. However, at high concentrations it may be toxic to organisms. The aim of the present study was to evaluate the fish adaptative response to experimental copper and cadmium pollution by biochemical methods, the enzymatic variations simultaneously appeared after acute copper and cadmium exposure. We studied effects of two essential and toxic metals (Cu and Cd) on enzymatic characteristics of the fish *Tilapia mossambicus* using environmentally relevant concentrations. Fish were exposed to metal through seawater for 24, 48,72and 96 hrs. Estimate the toxicity of cadmium and copper to fingerlings of *Tilapia mossambicus* as well as the effect of different concentrations of Cd and Cu on some enzymatic studies (Superoxide dis mutase, Catalase, Lactate dehydrogenase, glutathione-S-transferase, Protease, Amylase, lipase) in the fish. 96-h LC50s of Cu and Cd were 6.1 and 4.9 ppm, respectively. Levels of enzymes were increased within 2 days of exposure to muscle tissue. Activities of antioxidant and digestive enzymes such, Superoxide dismutase, Catalase, Lactate dehydrogenase, glutathione-S-transferase, were altered in exposed fish. The present study showed high toxicity of cadmium to fish *Tilapia mossambicus* comparing to copper.

INTRODUCTION

Toxicity tests using aquatic organisms play an important role in the development of proposals for environmental management and protection, especially for the aquaculture environment (Wall and Hanmer 1987; Hoi 2004). Trace metals such as Copper (Cu), Zinc (Zn), Cadmium (Cd) and Iron (Fe) were found to bioaccumulation in liver followed by gills and muscles in fish (Taylor *et al.*, 1985; Chan, 1995; Wong *et al.*, 1999; Somer, 2003; Ni *et al*., 2005). In addition, it is an important step to detect the levels of toxicants to be used in the experimental studies of the accumulation and effect of these toxicants to the marine organisms. There are many studies concern with the toxicity of cadmium on vertebrates and invertebrates (Rasmussen and Andersen, 2000, Adami *et al,* 2002 and Filiovic and Raspor, 2003). Fish exposed to high concentration of cadmium quickly develop lack of calcium and low blood hemoglobin. Microorganisms may suffer growth inhibition at cadmium concentration of 0.25 mg/l (Roberts, 2003).

The study of digestive enzymes in fish has a wide range of potential interest. Biochemical information about digestive enzyme equipment in fish can be related to their feeding habits and abilities, since whatever may be the food habit of the fish, adaptations of the digestive system of different species exhibit closer correlation with their diet than on their taxonomic category, allowing a more accurate evaluation of their specific role in aquatic ecosystems. On the other hand, the assessment of the activity of digestive enzymes in cultured species may be helpful in the selection of feed ingredients Lan and Pan, 1993.

Fish tissues, specifically the liver and kidney are endowed with an antioxidant defense systems to protect themfroman oxidative stress caused by metals (Basha and Rani, 2003; Atli et al., 2006; Atli and Canli, 2008a). Elevated levels of metals can induce oxidative stress by generating highly reactive oxygen species (ROS), such as hydrogen peroxide, superoxide radical and hydroxyl radical via Haber–Weiss and Fenton reactions that can oxidize proteins, lipids and nucleic acids, often leading to damage in cell structure or even cell death (Nagalakshmi and Prasad, 1998; Tripathi and Gaur, 2004; Dewez et al., 2005; Cao et al., 2010). Organisms have developed several protective mechanisms to remove ROS before the detrimental effects occur incell. Antioxidant enzymes, such as catalase (CAT), glutathioneperoxidase (GPX), glutathioneS transferase (GST), glutathionereduc-tase(GR) and superoxidedismutase (SOD) are of great importance in anoxidative stress to cope with fre eradicals leading several disturbances (Pinto etal.,2003;Tripathietal.,2006). The present work aimed to estimate the enzymatic changes on cadmium and copper exposure to fingerlings of fish *Tilapia mossambicus.*

MATERIALS AND METHODS

Fish management

Apparently healthy *Tilapia mossambicus* (3.5 ± 0.2 g) were obtained from local fish farm Pinnalore, Cudalore Dist, Tamilnadu, India. Prior to the experiment, fish were acclimatized for 2 weeks in 14 40-L glass aquaria under laboratory conditions (natural photoperiod 11.58–12.38 h); 10 fish per each aquarium. The continuous aeration was maintained in each aquarium using an electric air pumping compressors.

Analysis of the water physico-chemical variables

Water samples were collected from each aquarium prior to Cu and Cd exposure. Dissolved oxygen and temperature were measured on site with an oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). pH value was measured using a pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, USA). Total alkalinity and total hardness were measured according to Boyd (1984). The mean values for test water variables were as follows: dissolved oxygen 5.84±0.72 mg/L, pH 7.5±0.1, water temperature 25.5± 0.1^{o}C, total alkalinity 153.7±4.8 mg/L, and total hardness 222.5±2.9 mg/L.

Experimental procedures

The heavy metal Cu in the form of Copper chloride anhydrous (Merck, Mumbai, India) and Cd in the form of Cadmium chloride (Merck, Mumbai, India) was used in the present study. The acute toxicity test was performed for 4 days in which two replicates of seven different Cu and Cd concentrations (0, 2, 4, 6, 8 and 10 mg/L) were used (10 fish for each aquarium). At 24, 48, 72, and 96 h, fish dead were counted in the different Cu and Cd concentrations along with the control group. In this study, the acute toxic effects of Cu and Cd on *Tilapia mossambica* were determined by Behrens–Karber's method using the following formula (Klassen, 1991).

$$LC_{50} = LC_{100} \sum A \times B / N \text{ as mg/L}$$

Where LC50 and LC100 indicate the lethal doses for 50% and 100% of the tested fish. Value ‘‘A” gives the differences between the two consecutive doses, ‘‘B” the arithmetic mean of the mortality caused by two consecutive doses and ‘‘N” the number of tested fish in each group. The dead fish were removed immediately.

Enzyme assay

Homogenizing and centrifuging samples

The tissues were homogenized (1 : 10, w/v) in 20 mM Tris buffer (pH 7.8) containing 0.25 M sucrose and 1 mM EDTA at 9500 rpm for 3 min. Homogenates were centrifuged at 13000 g (Hettich Universal 30 RF) for 20 min at +4 °C and supernatant was used as enzyme source.

Catalase

CAT activity was assayed by the method of Chance and Machly (1955). The per fused liver and kidney were homogenated (10%) in 50 mM phosphate buffer, pH 7.0, and centrifuged at 16,000*g* for 45 min. The supernatant was used as the enzyme source. The reaction mixture contained 2 mL of phosphate buffer, pH 7.0, 0.45 mL H_2O_2, and 0.025 mL of enzyme source. The enzyme activity was expressed as micromoles of H_2O_2 metabolized/milligram protein/minute, at 250 nm of absorbance.

Super oxide dismutase

SOD activity was measured as the inhibition of photo reduction of nitroblue tetrazolium (NBT) by the enzyme as per the method of Beauchamp (1971). The perfused tissues were homogenized in (10% w/v) potassium phosphate buffer (pH 7.5) containing 1% polyvinyl pyrolidine and centrifuged at 16,000*g* for 15 min. The supernatant was used as the enzyme source. The total reaction mixture consisted of 100 mM phosphate buffer, 10 mM EDTA, 130 mM methionein, 750 mM NBT, 60 mM riboflavin, and enzyme source. The reaction was initiated by the addition of riboflavin, the samples were placed under fluorescence for 30 min, and the resulting color was read at 560 nm against a reagent blank kept in a dark place. The activity was expressed as units/milligram protein.

Glutathione S-transferase

GST activity was measured with its conventional substrate 1-chloro-2, 4-dinitrobenzene (CDNB), at 340 nm as per the method of Habig et al. (1974). The perfused tissues were homogenized in 50 mM Tris–HCl buffer, pH 7.4, and containing 0.2 M sucrose and centrifuged at 16,000*g* for 45 min at 4°C. The pellet was discarded and the supernatant was used as the enzyme source. The reaction mixture in a volume of 3 mL contained 2.4 mL of 0.3 M potassium phosphate buffer (pH 6.9), 0.1 mL of 30 mM CDNB, 0.1 mL of 30 mM GSH, and the enzyme source. The reaction was initiated by glutathione. The absorbance was read at 340 nm against a reagent blank and the activity was expressed as 1 μmol of thioether formed/milligram protein/minute.

Lactate dehydrogenase

Lactate dehydrogenase activity was measured according to Hansen and Sidell (1983) by observing the oxidation of NADH at 340 nm (pH 7.5). The assay mixture consisted of 50 mM imidazole, 1 mM KCN, and 0.15 mM NADH. The reaction was initiated by addition of 0.33 mM pyruvate. Since LDH displays substrate inhibition, pyruvate dose– response curves were carried out to determine the optimal concentration of pyruvate for each tissue. Activity is reported in international units (Amol NADH oxidized per minute) per milligram protein.

Amylase

Amylase activity was determined using the method of Rick (1984) with maltose as a standard, read at 550 nm on spectrophotometer. One unit of amylase activity was defined as number of micromoles of maltose released per minute per milligram of protein.

Proteases

Protease activity was assayed following the method of Eguchi and Iwamoto (1976) as outlined. 60 ml of enzyme sample was added with 200 ml aliquot of 1 % azocasein (in 0.2 m glycine – NaOH - pH 10.0) and incubated at 37oC for 30 mts. The reaction was terminated by the addition of 300 Aliquot of 5% trichlroacetic acid. After centrifugation at 1500g for 10 mts, an equal volume of 1M NaOH was added to the supernatant and absorbance was measured at 450 nm. One proteinaseunit was defined as the amount of enzyme that increased the absorbance by 1.0 OD under the given assay conditions.

Lipase

Lipase and activities were assayed according to the method of Pan and Wang (1997) using olive oil and sodium carboxyl methyl cellulose as substrate, respectively. Units of lipase and cellulose activities were considered as the number of micromoles of fatty acids and glucose released per minute per milligram of protein.

RESULTS

In the present study had derived maximum level of super oxide dismutase enzyme activity was recorded in 72 hrs value of 0.995 U/mg protein compare with control in cadmium exposure and minimum activity obtained in 24 hrs value of 0.354 U/mg protein compare with control in copper exposure (Fig. 1). Maximum level of Catalase enzyme activity was recorded in 72 hrs value of 3.632 U/mg protein compare with control in cadmium exposure and minimum activity obtained in 24 hrs value of 2.147 U/mg protein compare with control in copper exposure (fig. 2). Maximum level Lactate dehydrogenase of activity was recorded in 48 hrs value of 3.632 U/mg protein compare with control in cadmium exposure and minimum activity obtained in 24 hrs value of 1.404 U/mg protein compare with control in copper exposure (fig. 3). Maximum level Glutathione S-transferase of activity was recorded in 72 hrs value of 1.524 U/mg protein compare with control in copper exposure and minimum activity obtained in 24 hrs value of 0.834 U/mg protein compare with control in cadmium exposure (fig. 4). Maximum level of Protease activity was recorded in 72 hrs value of 2.97 U/mg protein compare with control in cadmium exposure and minimum activity obtained in 24 hrs value of 2.01 U/mg protein compare with control in copper exposure (fig. 5). Maximum level of Amylase activity was recorded in 48 hrs value of 9.60 U/mg protein compare with control in copper exposure and minimum activity obtained in 24 hrs value of 3.54 U/mg protein compare with control in cadmium exposure (fig. 6). Maximum level of Lipase activity was recorded in 96 hrs value of 10.56 U/mg protein compare with control in copper exposure and minimum activity obtained in 24 hrs value of 8.64 U/mg protein compare with control in copper exposure(fig. 7).

DISCUSSION

In general, the effect of heavy-metal exposure on the activity of glycolytic enzymes is controversial. Moreover, it depends on animal tissue and metal. For example, no change was found in PFK activity from both white and red muscles of Salmo trutta exposed to copper (0.08 μM.L−1; 10 °C, pH 5.0) (Beaumont *et al*., 2000); but the activity was reduced in the liver and white muscles and increased in the red muscles of Oreochromis niloticus exposed to cadmium (1–14 μM $CdCl_2 \cdot L^{-1}$, 24 °C, pH 7.6) (Almeida *et al*., 2001). Cyprinus carpio exposed to copper increased LDH activity in the liver, heart and gills (Tóth *et al*., 1996), while the exposure of Sparus auratus to this metal led to a decrease in LDH activity in the liver (Antognelli *et al*., 2003). Exposure to cadmium increased LDH activity in liver, heart and gills of Mugil cephalus (Hilmy *et al*., 1985) as well as in red muscles of O. niloticus, whose liver and white muscles were not affected by the metal (Almeida *et al*., 2001).

Transition metals act as catalysts in the oxidative reactions of biological macromolecules, though their toxicities may depend upon the oxidative tissue damage. Redox active metals such as copper, chromium and iron, undergo redox cycling, whereas redox-inactive metals, such as cadmium, lead and mercury deplete major antioxidants in the cell, especially thiol containing antioxidants and enzymes. Both of these metals can cause significant increases in an ROS production, followed by a situation known as ‘‘oxidative stress’’ leading various dysfunctions in lipids, proteins and DNA (Ercal *et al*., 2001; Pinto *et al.,* 2003).

Previous studies and also this study suggest that antioxidant enzymes have gained an importance in preventing the hazardous effects of metals, as they could be warning signals for severe damage to aquatic environment or organisms living in. One of the major and well known multifactorial mechanisms is a production of reactive oxygen species induced by metals. Fenton like reactions play a significant role in the oxidative stress, caused by redox active metals. On the other hand, an oxidative stress can deplete the sulfhydryl content indirectly by redox-inactive metals. In this mention, to investigate the differences between redox active and inactive metal effects on antioxidant enzymes in different tissues gain significance for ecotoxicological researches. Therefore, the metals in this study are selected in regard to their essentiality in fish metabolism and redox characteristics to evaluate better their behaviors in an antioxidant response. Considering importance of an antioxidant system for animal metabolism and also potential effects of heavy metals on them led this study to be undergone, taking into account of different exposure protocols.

As of metal species and exposure protocol, tissue type also played significant roles from metal stress as the liver enzymes were more sensitive than kidney enzymes. This study also demonstrated the role of the liver in an antioxidant enzyme response as a result of its higher sensitivity to metals comparing to the kidney. This is possibly because of the liver being the main contribution of an ROS. The liver is known to be a stronger organ into the face of oxidative stress than the other tissues and also a uniform organ with the 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 (G¨ul *et al*., 2004; Avci *et al*., 2005). GST and CAT activities, after acute Cu and GPX activities, after an acute Cr exposure were totally inhibited, suggesting sensitivity of antioxidant enzymes to acute exposures. The induction of elevated levels of SOD, xanthine oxidase and GPX with a simultaneous increase in the levels of GST and CAT shows a possible shift towards detoxification mechanismunder long-term exposure cadmium. GST and CAT activities, after acute Cu and GPX activities, after an acute Cr exposure were totally inhibited, suggesting sensitivity of antioxidant enzymes to acute exposures. The induction of elevated levels of SOD, xanthine oxidase and GPX with a simultaneous increase in the levels of GST and CAT shows a possible shift towards a detoxification mechanism under long-term exposure cadmium. Similar activity levels were also reported in laboratory animals under copper stress (Basha and Rani, 2003).

An evident for this Basha and Rani (2003) suggested that higher hepatic GST activity than the activity of kidney GST in O.mossambicus exposed to Cd may depend upon the effective role of liver in detoxification, which is also in agreement with our data obtained from acute metal exposures. Moreover kidney seemed to be the main site for GPX for the authors, is in accordance with our results exhibiting the enhancement of the GPX activity only in the kidney tissue after chronic Cd and Cu exposures. In addition, Tagliari *et al*. (2004) found an increase in an SOD activity after acute exposure of Cr (VI) in O. niloticus; however, the CAT activity did not change. It was known that Cr (VI) is transformed into Cr (V) in the liver, and the hydroxyl lradical formed as a result of this process via the Fenton –Haber–Weiss reaction. Nevertheless, an increased SOD activity and decreased CAT activity after an acute Cr (VI) exposure might be associated with the excess production of hydrogen peroxide by high SOD activity, which therefore may lead a reduction in the CAT activity. Similar results were also obtained from several fresh water fishes exposed to Cd acutely (Palace and Klaverkamp, 1993; Hansenetal., 2006).

Studies of metal-induced alterations in antioxidant enzyme activities reported that especially SOD and CAT, two major antioxidant enzymes, are affected by Cd both in vitro and in vivo (Almeida *et al*., 2002; Atli *et al.,* 2006; Hansen *et al*., 2006). For instance, Zikic *et al*. (2001) found decreased activity of SOD in erythrocytes of Carassius auratusgibelio Bloch. during acute exposure to Cd in a time course manner, which indicated the presence of ROS-induced peroxidation leading to the destruction of erythrocytes membranes .It was also indicated that differential responses of Cd may be related to biomarkers such as increased antioxidant enzymes, of oxidative stress defining as the imbalance between oxidant fluxes and the antioxidant defenses (Almeida *et al*., 2002). A decline in GR activity may result in GSH depletion if extra synthesis of GSH cannot take place to preserve its redox status, as a result of pro oxidative effects. On the other hand, enhancement of GR activity could be occurred due to re-establishment of the GSH levels that is oxidized. Matos *et al.* (2007) suggested that it would be normal to observe an increase in GR activity in an oxidative stress situation. It is also emphasized that variation in the GST activity seems to be accompanied by GSH depletion in metal exposed organisms (Elia *et al*., 2003).

Phase II enzyme systems such as GST facilitates conjugation of electrophilic substances or groups to tri pipetide glutathione in order tomake the xenobiotics more hydrophilic for transportation or excretion (Ozmen *et.al*. 2006). Dautremepuits *et al*. (2004) were observed a decrease in antioxidant enzyme activity in the liver of Cyprinus carpio exposed to Cuand they indicated that excess Cu causes rapid GSH oxidation evenat low non toxic Cu concentrations in hepatocytes followed by GST depletion from the previous researches which is also in accordance with present data. In addition, the present results suggest that GST insensitive to products of Haber–Weiss reaction, though significant GST and GPX inductions were recorded in the liver of O. niloticus after chronic Cd exposure. This increase may mean the high production of GSSG (Zirong andShijun, 2007). An increase in GPX activity was also recorded in this study in the kidney after chronic Cd exposure. In vitro and *invivo* studies demonstrated that Cu can act both directly through binding to–SH groups and indirectly by inducing oxidative stress (Viarengo 1993; Atliand Canli, 2008a).The authors indicated that organ-specific changes might be related to the exposure route, mode of entry and chemical uptake and bioaccumulation by the organs gives support to our data also (Ahmad *et al.,* 2005).

FIGURES

Fig. 1 Super oxide dismutase (U/mg)

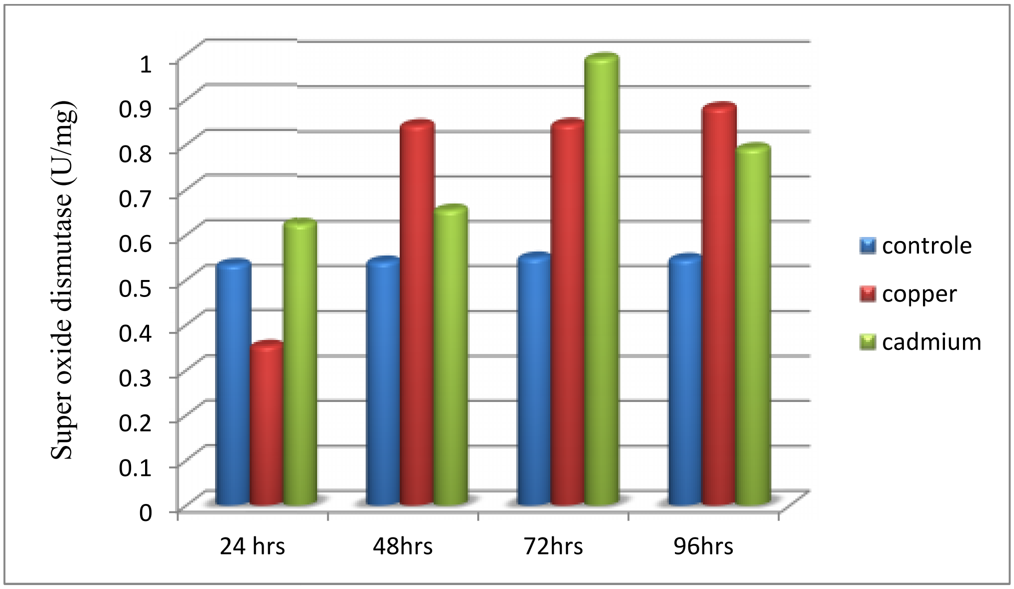

Fig. 2 Catalase activity (CAT) (U/mg)

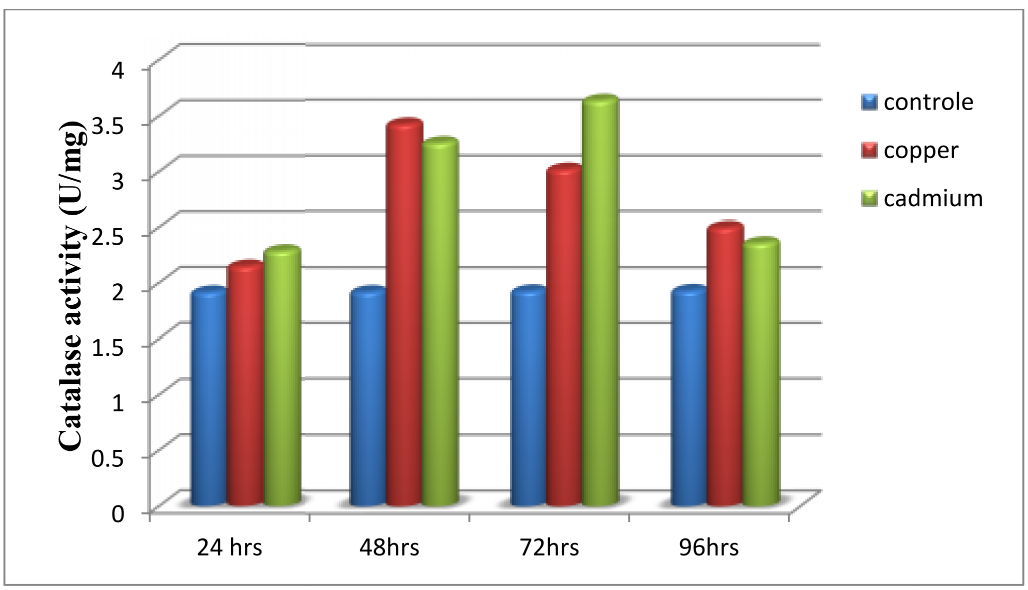

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Fig. 3 Lactate dehydrogenase activity (LDH) (U/mg)

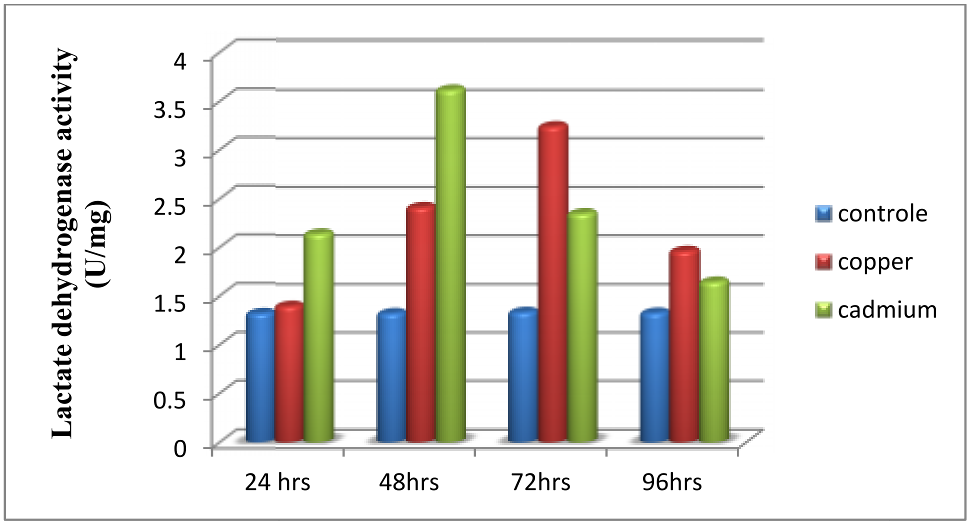


Fig. 4 Glutathione S-transferase activity (GST) (U/mg)

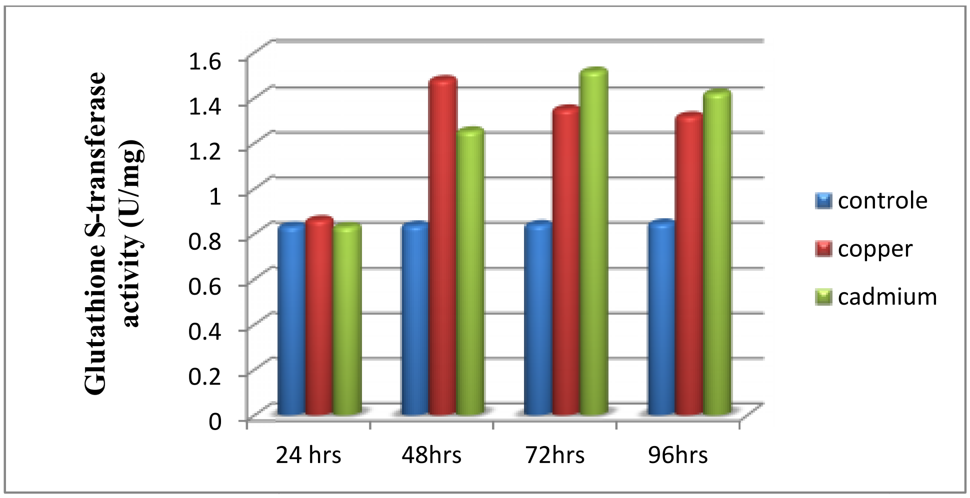


Fig. 5 Protease activity (U/mg)

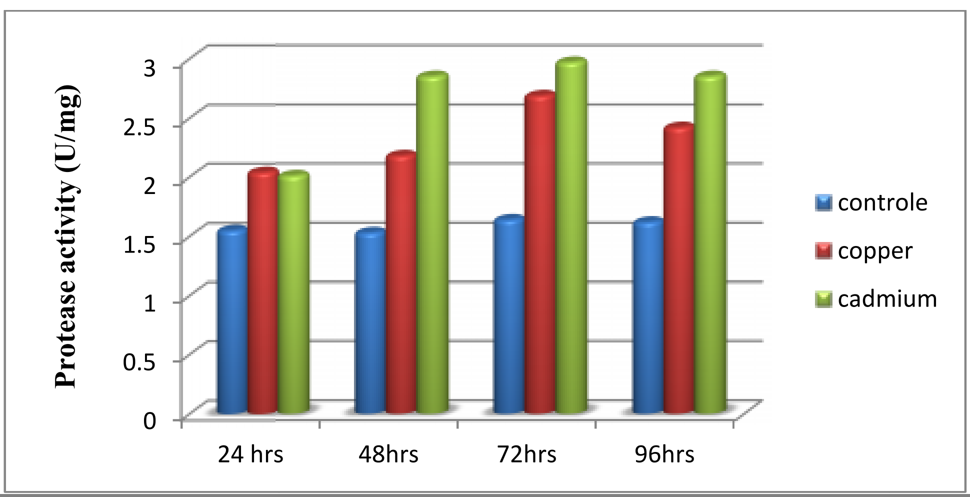


Fig. 6 Amylase activity (U/mg)

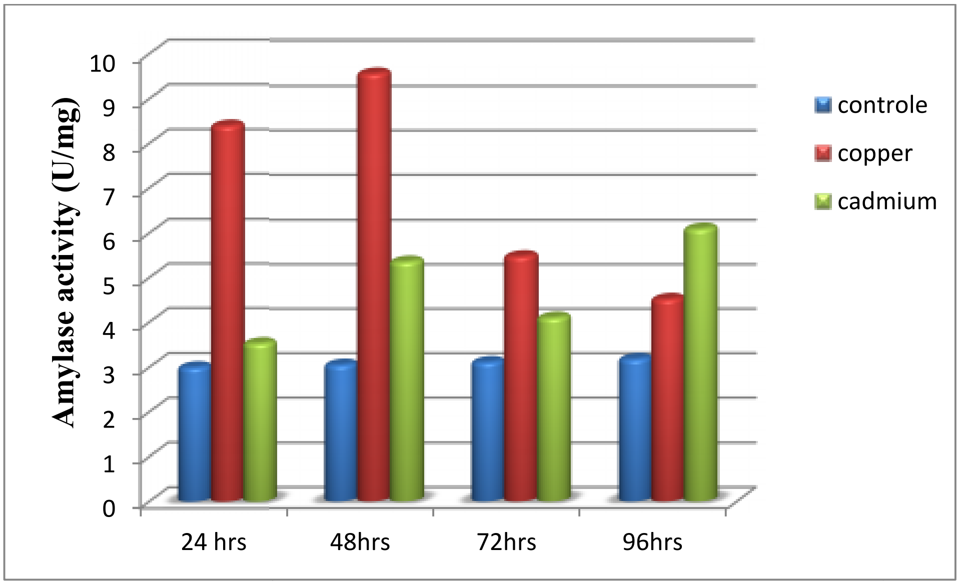
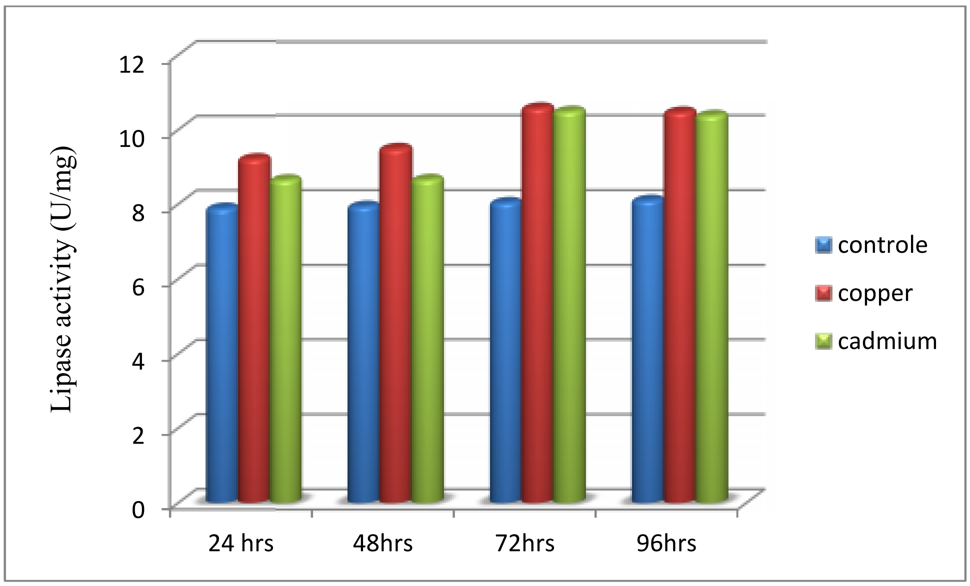


Fig. 7 Lipase activity (U/mg)



REFERENCES

1. Adami, G.m Barbieri, P., Fabiani, M., Piselli, S., Predonzani, S. and Reisenhofer, E. (2002).Levels of cadmium and zinc in hepatopancreas of reared *Mytilus galloprovincialis* from the Gulf of Trieste (Italy). Chemosphere, 48: 671-677.
2. Almeida, J.A., Novelli, E.L.B., Dal Pai Silva, M., Alves Junior, R., 2001. Environmental cadmium exposure and metabolic responses of the Nile tilapia Oreochromis niloticus. Environ. Pollut. 114, 169–175.

3. Antognelli, C., Romani, R., Baldracchini, F., De Santis, A., Andreani, G., Talesa, V., 2003. Different activity of glyoxalase system enzymes in specimens of Sparus auratus exposed to sublethal copper concentrations. Chem. Biol. Interact. 142, 297–305.
4. Atli, G.,Alptekin,O¨., T ¨ ukel, S.,Canli,M.,2006.Responseofcatalaseactivityto Ag+, Cd2+, Cr6+, Cu2+ and Zn2+ in fivetissuesoffreshwaterfish Oreochromis niloticus. Comp.Biochem.Physiol.C143, 218–224.
5. Atli, G., Canli, M., 2008a.Responsesofmetallothioneinandreducedglutathionein a freshwater fish Oreochromis niloticus following metalexposures.Environ. Toxicol. Pharm.25, 33–38.
6. Basha P.S. and A.U. Rani, 2003. Cadmium induced antioxidant defense mechanism in freshwater teleost Oreochromis mossambicus (Tilapia). Ecotoxicol. And Environ. Safety. 56(2): 218-221.
7. Beaumont,M.W., Butler, P.J., Taylor, E.W., 2000. Exposure of brown trout, Salmo trutta, to a sub-lethal concentration of copper in soft acidic water: effects upon muscle metabolism and membrane potential. Aquat. Toxicol. 51, 259–272.
8. Cao, L., Huang, W., Liu, J., Yin, X., Dou, S., 2010. Accumulation and oxidative stress biomarkers in Japanese flounder larvae and juveniles under chronic cadmium exposure. Comp. Biochem. Physiol. C 151, 386–392.
9. Chance, B. and Maehly, A. C. (1955) Assay of catalase and peroxidase. *Methods Enzymol.* 2, 764-775.
10. Dautremepuits, C., Paris-Palaciosa, S., Betoullea, S., Vernet, G., 2004.Modulation in hepatic and head kidney parameters of carp (Cyprinus carpio L.) induced by copper and chitosan. Comp. Biochem. Physiol. C 137, 325–333.
11. Dewez, D.,Geoffroy,L.,Vernet,G.,Popovic,R.,2005.Determinationof photosynthetic and enzymatic biomarkers sensitivity used to evaluate toxic effects of copper and fluid oxonilin alga Scened esmus obliquus. Aquat.Toxicol. 74, 150–159.
12. Elia, A.C.,Galarini,R.,Taticchi,M.I.,D¨ orr, A.J.M.,Mantilacci,L.,2003.Antioxidant responses and bioaccumulation in Ictalurus melas under mercury exposure. Ecotoxicol. Environ. Saf. 55,162–167.

13. Filipovic, V. and Raspor, B. (2003). Metallothionein and metal levels in cystol of liver, kidney and brain in relation to growth parameters of *Mullus surmuletus* and *Liza aurata*. From the Eastern Adriatic Sea. Water Research. 37: 3253- 3262.
14. Hansen,B.A.,Romma,S.,Garmo,O.A.,Olsvik,P.A.,Andersen,R.A.,2006.Antioxidative stress proteins and their gene expression in brown trout (Salmo trutta) from three rivers with different heavymetal levels.Comp.Biochem.Physiol.C143, 263–274.
15. Hilmy, A.M., Shabana, M.B., Daabees, A.Y., 1985. Effects of cadmium toxicity upon the in vivo and in vitro activity of proteins and five enzymes in blood serum and tissue homogenates of Mugil cephalus. Comp. Biochem. Physiol. C 81, 145–153.
16. Klassen, C.D. 1991. Principles of toxicology. In: Gilman, A.G., Tall, T.W., Nies, A.S., Taylor, P. (Eds.), Pharmacological Basis of Therapeutics, eighth ed. McGraw-Hill, Berlin, pp. 49–61.
17. Lan, C.C., Pan, B.S., 1993. In vitro digestibility simulating the proteolysis of feed protein in midgut gland of grass shrimp Ž*Penaeus monodon*. Aquaculture 109, 59–70.
18. Matos P.,Fontainhas-Fernandes A.,Peixoto,F.,Carrola,J.,Rocha,E., 2007. Biochemical and histological hepatic changes of Niletilapia Oreochromis niloticus exposed to carbaryl pest. Biochem. Physiol.89, 73–80.
19. Nagalakshmi N., 1998. Copper-induced oxidative stress in Scenedesmus bijugatus: protective role of free radical scavengers. Bull.Environ.Contam.Toxicol.61,623–628.
20. Ni, I.H., Chan, S.M. and Wang, W.X. 2005. Influences of salinity on the biokinetics of Cd, Se, and Zn in the intertidal mudskipper *Periophthalmus cantonensis*. Chemosphere, 61: 1607-1617.
21. Ozmen, M.,Gungordu, A.,Kucukbay, F.Z.,G uler, R.E.,2006.Monitoring the effects water pollution Cyprinus carpio in Karakaya Dam Lake.Turkey.
22. Palace, V.P., Klaverkamp, J.F., 1993. Variation of hepatic enzymes in three species of freshwater fish from Precambrian shield lakes and the effect of cadmium exposure. Comp. Biochem. Physiol. C 104, 147–154.
23. Pinto, E., Sigaud-Kutner, T.C.S., Leitao, M.A.S., Okamoto, O.K., Morse, D., Colepicolo, P., 2003. Heavy metal-induced oxidative stress in algae. J. Phycol. 39, 1008–1018.

24. Rasmussen, A. D. and Andersen, O. (2000). Effects of cadmium exposure on volume regulation in the lugworm, *Arenicola marina*. Aquatic toxicology. 48: 151-164.
25. Roberts, M. (2003). Review of risks from metals in the U K. Chemical Stakcholder Forum, Fourteenth meeting, pp 20.
26. Somero, G.N., 2004. Adaptation of enzymes to temperature: searching for basic “strategies”. Comp. Biochem. Physiol. B 139, 321–333.
27. Tagliari, K.C., Vargas, V.M.F., Zimiani, K., Cecchini, R., 2004. Oxidative stress damage in the liver of fish and rats receiving an intraperitoneal injection of hexavalent chromium as evaluated by chemiluminescence. Environ. Toxicol. Pharm. 17, 149–157.
28. Taylor, D., Maddock, B. and Mance, G. 1985. The acute toxicity of nine "grey list" metals (arsenic, boron, chromium, copper, lead, nickel, tin, vanadium and zinc) to two marine fish species: dab (*Limanda limanda*) and grey mullet (*Chelon labrosus*). Aquatic Toxicology, 7: 135–144.
29. Tóth, L., Juhász, M., Varga, T., Nemcsók, J., 1996. Some effect of CuSO4 on carp. J. Environ. Sci. Health, Part B, Pestic. Food Contam. Agric. Wastes 31, 627–635.
30. Tripathi, B.N., Gaur, J.P., 2004.Relationshipbetweencopper-andzinc-induced oxidative stress and proline accumulation in Scenedesmus sp.Planta219, 397–404.
31. Tripathi, B.N., Mehta, S.K., Amar, A., Gaur, J.P., 2006.Oxidative stress in *Scenedesmus* sp. during short-and long-term exposure to Cu2+ andZn2. Chemosphere62, 538–544.
32. Viarengo A., Nott J., 1993, Mechanisms of heavy metal cation homeostasis in marine invertebrates. Comp. Biochem. Physiol. Comp. Pharmacol. Toxicol. 104: 355–372.
33. Wall TW and Hanmer RW. 1987. Biological testing to control toxic water pollutants. Journal of the Water Pollution Control Federation 59(1), 7 – 12.
34. Wong, P.P.K., Chu, L.M. and Wong, C.K. 1999. Study of toxicity and bioaccumulation of copper in the silver sea bream *Sparus sarba*. Environment International, 25(4): 417-422.
35. Zirong, X., 2007.Effects of waterborne Cd exposure on glutathione metabolism in Niletilapia (Oreochromis niloticus) liver. Ecotoxicol. Environ.Saf. 67, 89–94.