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HEAVY METAL INDUCED ENZYME RESPONSE IN *TILAPIA MOSSAMBICUS*

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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 the mfroman 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 & 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.

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 mossambicus* were determined by Behrens–Karber's method using the following formula (Klassen, 1991).

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 perfused tissues were homogenate (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 and Fridovich (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 thio ether 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 and Stegbauer (1984) with maltose as a standard and read at 550 nm on a spectrophotometer. One unit of amylase activity was defined as the 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 protein as e unit 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 AND DISCUSSION

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 (Figure 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 (Figure 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 (Figure 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 (Figure 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 (Figure 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 (Figure 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(Figure 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 CdCl2·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 (Pinto *et al.,* 2003).

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.

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 mechanism under 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; Hansen etal., 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 to make 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 and Nott, 1993; Atli and 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).

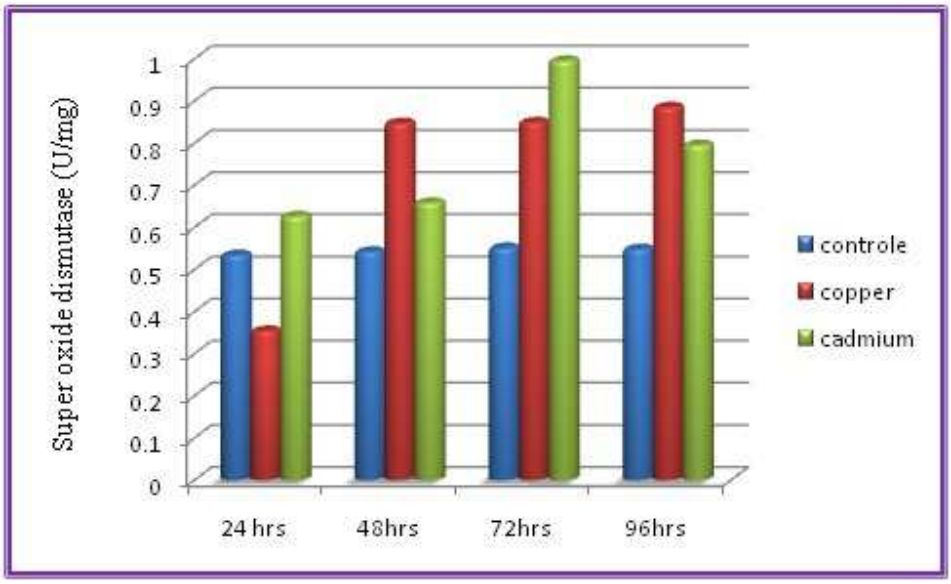

Figure 1 Super oxide dismutase (U/mg)

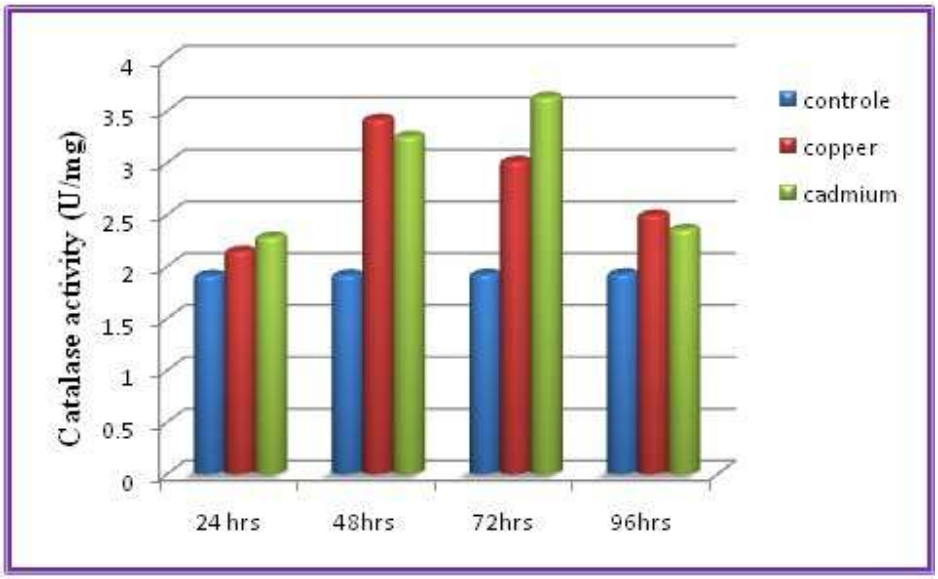

Figure 2 Catalase activity (CAT) (U/mg)

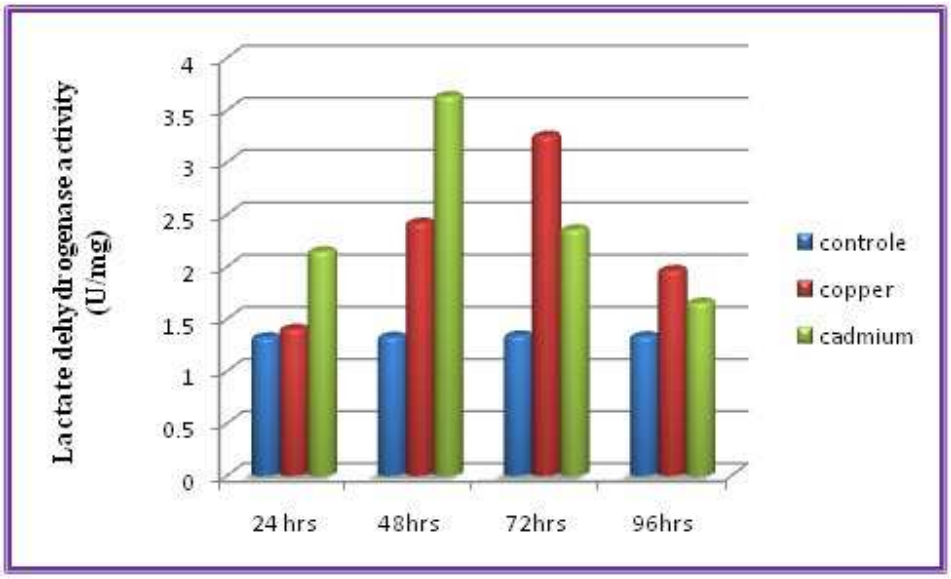

Figure 3 Lactate dehydrogenase activity (LDH) (U/mg)

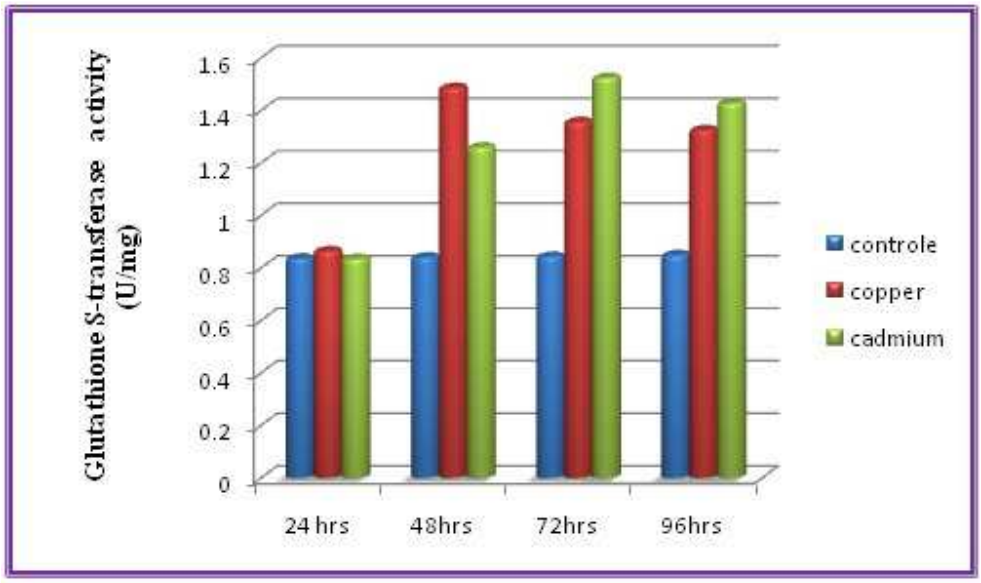

Figure 4 Glutathione S-transferase activity (GST) (U/mg)

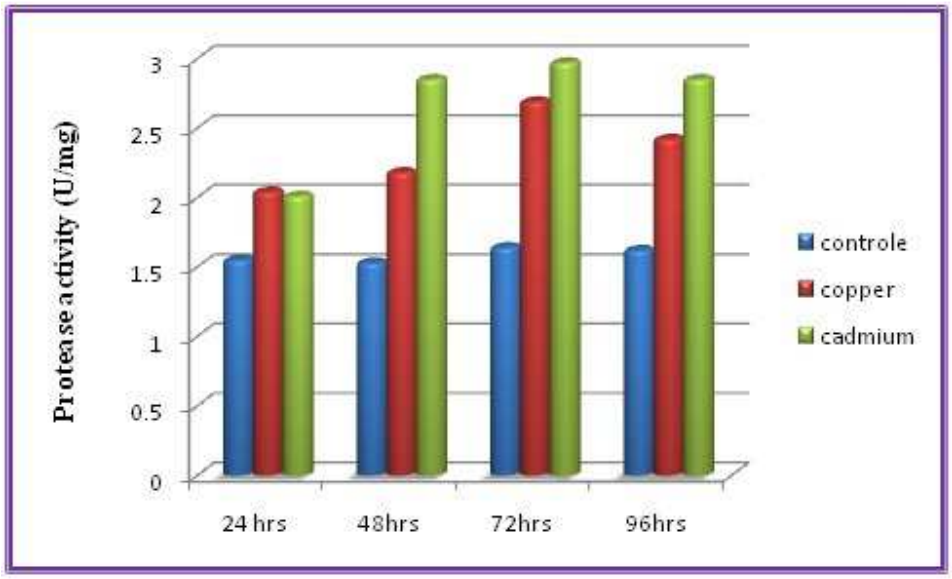

Figure 5 Protease activity (U/mg)

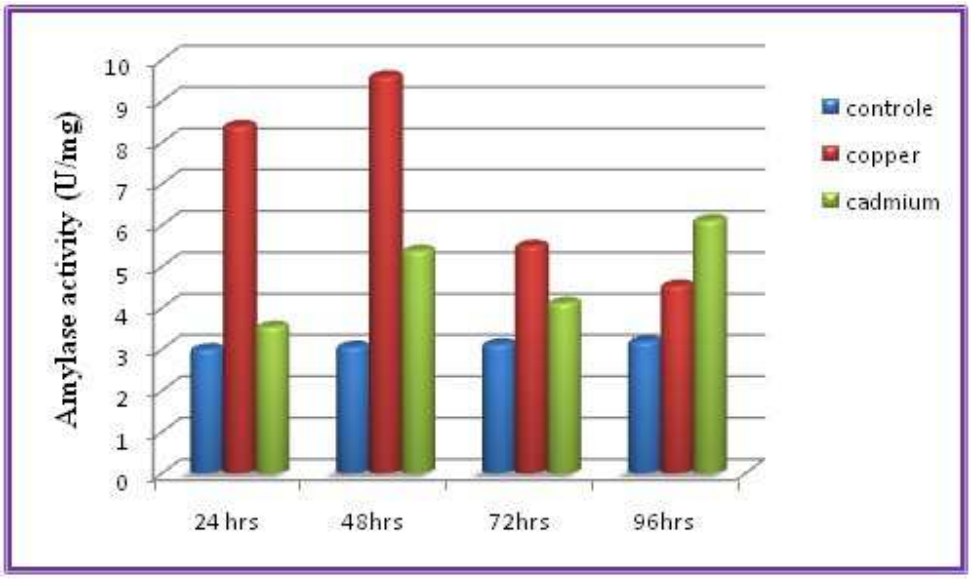

Figure 6 Amylase activity (U/mg)

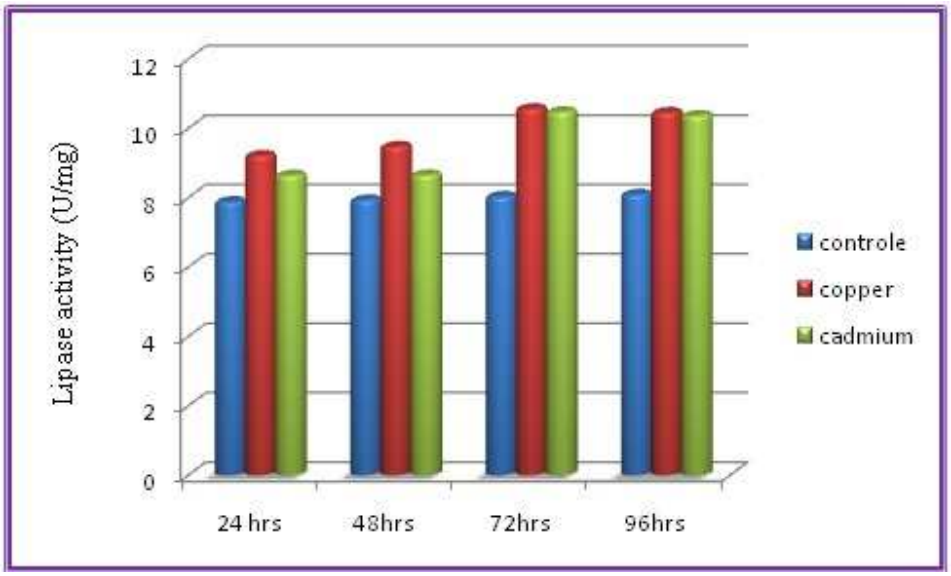

Figure 7 Lipase activity (U/mg)

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