Poll Res. 40 (May Suppl. Issue) : S219-S231 (2021)

ISSN 0257–8050

EFFECT OF HEAVY METALS (LEAD AND CADMIUM) ON THE ACTIVITY OF ANTIOXIDANTS AND METABOLIC ENZYMES IN *OREOCHROMIS NILOTICUS*: A COMPARATIVE AND SYNERGISTIC STUDY

CINNIE SUSAN ANTONY AND A. P. THOMAS

School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala.

(Received 1 November, 2020; accepted 15 February, 2021)

ABSTRACT

Presence of heavy metals in aquatic environment induces stress in aquatic life. Present study has been taken up to assess the effect of two heavy metals (lead and cadmium), individually and in combination, on a freshwater fish *Oreochromis niloticus*. The study aimed to evaluate the impact of the heavy metals (sublethal dose) on the activity of two antioxidants, catalase (CAT) and superoxide dismutase (SOD) and two metabolic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the liver, gills, muscle and kidney of the fish after one week and three weeks exposure to heavy metals. Differential variations were observed in the activity of antioxidant enzymes (CAT & SOD) as well as the metabolic enzymes (AST & ALT) in the organs when applied individually as well as in mixture (lead + cadmium). It was found that the impact of the metals is more significant when applied in combination rather than individually indicating the synergistic effect. It was noted that more significant changes occurred in the liver, the major metabolic organ of the fish compared to gills, muscle and kidney. The study thus tries to illustrate the potential of the above mentioned antioxidants and metabolic enzymes as biomarkers in toxicological studies.

KEY WORDS : Heavy metals, Antioxidants, Metabolic enzymes, Synergistic effect.

INTRODUCTION

The aquatic ecosystem is mostly the ultimate recipient of many waste substances including heavy metals (Malik *et al*., 2010). Presently, there is an increasing concern world over on the indiscriminate discharge of heavy metals resulting in environmental pollution and toxicity risk to non-target organisms. Sublethal pollution of heavy metal in the aquatic environment causes chronic stress conditions on aquatic life (Sary and Beheshti, 2011). Evaluation of heavy metal toxicity is necessary as its high concentration could predispose humans to health risk. Copper, zinc, nickel, chromium, cadmium and lead are listed as priority hazardous pollutants in many countries because of their toxicity, persistence, and affinity for bioaccumulation (US EPA, 2009).

In the aquatic environment, cadmium is one of the most hazardous elements among the non-essential heavy metals (UNEP, 1985). Due to the interactions with environmental variables and other toxic agents, biological effects of cadmium in aquatic organisms are complex. Cadmium has a cumulative polluting effect even at sublethal concentration. It causes serious disturbances in metabolism of aquatic organisms which leads to abnormal behaviour, locomotion anomalies or anorexia (Cicik and Engin, 2005).

Lead, a prominent toxicant also shows toxic effects on living beings upon exposure (Ercal *et al*., 2001; Pande and Flora, 2002; Patrick, 2006). Aquatic animals absorb lead from surrounding water, binds to erythrocytes and pass it to body organs such as liver, kidney, heart, spleen and muscle via blood (Meyer *et al*., 2008). Though the mechanism of lead

toxicity is not fully understood, it is however proved that lead causes oxidative stress by stimulating the generation of reactive oxygen species (ROS) (Dewanjee *et al*., 2013).

There is considerable research on the effects of exposure of aquatic organisms to a particular contaminant (Nabinger *et al*., 2018; and Zheng *et al*., 2016b). But in the natural environment , massive exposure to a single pollutant is rare. The aquatic organisms are exposed rather to multiple chemicals and not just to a single pollutant (Boulanger *et al*., 2019; Fang *et al*., 2019; Feo *et al*., 2010; Lao *et al*., 2010; Turgut, 2003). But there is limited works on the effect of metal mixtures on organisms. Exposure to a particular metal or to metal mixtures may lead to several toxic effects in animals like tissue damage, respiratory changes, alterations of biochemical and physiological mechanisms, and ultimately mortality (Heath, 1995). Therefore, the enzymatic and non-enzymatic parameters gain importance as sensitive tools to estimate the effects of single metal and metal mixture exposures before the occurrence of hazardous effects in organisms.

Antioxidant enzymes play a crucial role in maintaining cellular homeostasis. Heavy metals transform O_2 ˙into reactive oxygen species (ROS), which are highly toxic and mutagenic. Antioxidant enzymes, such as catalase (CAT) and superoxide dismutase (SOD) act by detoxifying the reactive oxygen species (ROS) generated. Therefore, antioxidant enzymes are considered as sensitive biomarkers in environmental stress before hazardous effects occur in fish, and are important parameters for testing water for the presence of toxicants (Heath, 1987; and Geoffroy *et al*., 2004). Metabolic enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the most important enzymes acting as transferases involved in amino acid metabolism and they are known to be sensitive to metal exposures (Levesque *et al*., 2002; Gravato *et al*., 2006).

A validated approach for early warning of metal pollution is the analysis of enzyme biomarkers in aquatic organisms, particularly in fish (Van der Oost *et al*., 2003; and Osman *et al*., 2010). During stress conditions fishes change and adapt their metabolic functions (Malarvizhi *et al*., 2012). Inhibition or induction of the enzymes such as AST and ALT and antioxidants like CAT and SOD can be used to indicate tissue damage (Nemcsok and Boross, 1982; Webb *et al*., 2005).

The fish species *Oreochromis niloticus* has been widely used in environmental studies as well as in evaluating the toxicity of contaminants in aquatic ecosystems (Almeida *et al*., 2001).

The present work tries to throw light on the synergistic effect of metal mixture (lead and cadmium) in comparison to single metal exposure on the fish *Oreochromis niloticus*.

MATERIALS AND METHODS

Experimental animal and maintenance

Healthy specimens of fresh water fish *Oreochromis niloticus* (size 9 ± 2 cm; weight 22± 2 gm) were procured from fish farm at Aymanam, Kottayam District, Kerala. They were acclimatized to laboratory conditions in cement tanks in dechlorinated water at (25 ± 2 °C) for 2 weeks. Fishes were fed with commercially available fish feed during acclimatization. Three fourth of the water was renewed daily to avoid accumulation of excretory waste. Physicochemical characteristics of the water used for the experiments were analyzed by standard methods (APHA, AWWA, WPCF, 2005). Absence of heavy metals, lead and cadmium were confirmed in the water and also in the fish feed used for the experiment.

Acute Toxicity assay

Stock solution of lead nitrate (1 g/l) and cadmium chloride (1 g/l) was prepared using analytical grade lead nitrate and cadmium chloride (Merck). Toxicity tests have been performed in accordance with the standard methods (APHA, 2005) to find out 96 hr LC_{50} for lead nitrate and cadmium chloride separately. The stock solution was freshly prepared which was renewed after every 24 hours. The fishes were exposed to increasing concentrations (5 mg/l to 85 mg/L) of lead nitrate and (5 mg/L to 55 mg/L) cadmium chloride. On the basis of fish mortality in each tank performing static bioassay test, 96 hr LC50 was calculated by Finney's Probit analysis method and SPSS Statistical Software. The 96 hr LC 50 was found to be 43.35 mg/l and 25.25 mg/l for lead nitrate and cadmium chloride respectively.

Sublethal studies

One tenth (1/10) of the 96 hr LC_{50} value was taken as test dose (Sprague, 1971). Fishes were divided into four groups of 20 each. The first group was exposed to 1/10 LC_{50} dose (4.33 mg/l) of lead

nitrate; the second group to 1/10 LC_{50} (2.52 mg/L) of cadmium chloride; the third group was exposed to a mixture of lead nitrate and cadmium chloride (both 1/10 their respective LC_{50}); the fourth group was taken as control. Desired concentration of the toxicant was added into each experimental tank after removal of the same volume of water and renewed daily in order to maintain a constant concentration. At the end of the stipulated period (1 week and 3 weeks) of exposure, fishes were randomnly selected (10 from each group) and sacrificed for further studies.

Assay of Antioxidants [Catalase (CAT) and Superoxide dismutase (SOD)]

The activity of CAT was measured (Aebi, 1984) in the liver, gill, kidney and muscle at the end of 1 week and 3 weeks. The values were expressed as mmoles H_2O_2 decomposed/mg/min. SOD activity in the different tissues (liver, gill, kidney and muscle) were analysed (Kakkar *et al.*, 1984) and expressed as U/g of protein.

Assay of metabolic enzymes [Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)]

The activity of AST and ALT was determined using the method of Reitman and Frankal (1957) in the liver, gills, muscle and kidney at the end of 1 week and 3 weeks. The values were expressed as µm/g and nmole/min/mg respectively.

Statistical analysis of the data

One way analysis of variance (ANOVA) was applied to compare the means between different groups to test the level of significance. The values for all the biomarkers are expressed as mean ± S.E. (n=6). Two way ANOVA was done to compare the effect on different organs and between different metal exposures. Student t test was performed to compare between weeks.

RESULTS

Changes in CAT activity

In the present study the catalase activity increased significantly (P<0.05) in liver in all treatments compared to control after one week of exposure (Table 1 and Fig. 1). However in the lead + cadmium (mixture) treated group, the increase was highest, followed by cadmium treated and least in the lead treated group. In the kidney the catalase activity increased in all treatments. But there was significant increase (P<0.05) only in mixture treated group and cadmium treated group. In the lead treated group the increase was insignificant. In the gills and muscle there was a significant decrease in catalase in all the treatments (Fig. 1a).

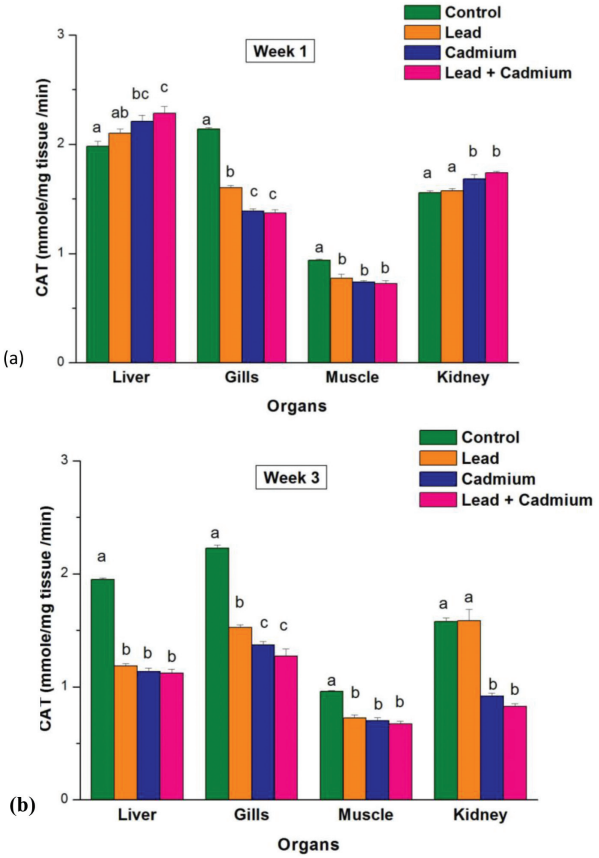

Fig. 1. Effect of lead and Cadmium on catalase (CAT) activity in different organs of fish, Oreochromis niloticus

At the end of three weeks of exposure there was significant decrease (P<0.05) in catalase activity in all the organs in all treatments (Table 1 and Fig.1b). However the decrease was highest in mixture treated group compared to cadmium or lead only treatments. The results show that metal mixture has a greater impact on catalase activity.

Changes in SOD activity

After one week of exposure, SOD activity increased in all the organs in all treatments. Metal mixture showed the highest increase in SOD activity in all organs compared to other treatments. Cadmium treated group had greater impact on SOD activity than lead in all the organs (Table 2 and Fig. 3a). At

Table 1. Effect of lead and cadmium on catalase (CAT) activity in different organs of the fish, *Oreochromis niloticus*

Groups	Catalase (mmole/mg tissue/min)			
	Liver	Gills	Muscle	Kidney
		Week 1		
Control	$1.98 \pm 0.045^{a,A}$	$2.13 \pm 0.014^{a,A}$	$0.94 \pm 0.008^{b,A}$	$1.55 \pm 0.016^{a,A}$
Lead	$2.10 \pm 0.038^{ab,B}$	$1.60 \pm 0.022^{b,B}$	$0.77 \pm 0.039^{a,B}$	$1.57 \pm 0.023^{a.B}$
Cadmium	$2.20 \pm 0.056^{bc,C}$	$1.38 \pm 0.021^{c,C}$	$0.738 \pm 0.013^{a,C}$	$1.68 \pm 0.037^{b,C}$
Lead + Cadmium	$2.28 \pm 0.063^{c,C}$	$1.36 \pm 0.032^{c,C}$	$0.726 \pm 0.027^{a,C}$	$1.74 \pm 0.014^{b,C}$
		Week 3		
Control	$1.94 \pm 0.015^{a,NS,A}$	$2.22 \pm 0.025^{a,NS,A}$	$0.959 \pm 0.007^{a,NS,A}$	$1.57 \pm 0.029^{a,NS,A}$
Lead	$1.18 \pm 0.021^{b,*,B}$	$1.52 \pm 0.024^{b,NS,B}$	$0.725 \pm 0.027^{b,NS,B}$	$1.58 \pm 0.104^{a,NS,B}$
Cadmium	$1.13 \pm 0.031b^{*,C}$	$1.37 \pm 0.030^{c,NS,C}$	$0.701 \pm 0.026^{b,NS,C}$	$0.918 \pm 0.027^{b,*,C}$
Lead + Cadmium	$1.12 \pm 0.033^{b,*,C}$	$1.27 \pm 0.065^{c,NS,C}$	$0.672 \pm 0.023^{b,NS,C}$	$0.826 \pm 0.024b,^{*,C}$

the end of three weeks of exposure, all treatments showed significant increase (P<0.05) than control in all the organs. The lead + cadmium treated group was found to have highest increase in SOD levels than other groups in all the organs (Table 2 and Fig. 3b).

According to the two way analysis of variance (two way ANOVA), exposure to lead+cadmium mixture has greater impact on SOD activity compared to lead or cadmium alone. Besides, SOD levels in all. organs of week 3 were found significantly different from week 1 (Fig. 4a-d).

Changes in activity of AST and ALT

The levels of AST and ALT in liver, gills, muscle and kidney increased in all treatments compared to control after one week of exposure (Table 3 and 4). In cadmium treated group and lead+ cadmium group, a significant increase (P<0.05) in AST was observed in liver. However, in lead+cadmium treated group highest AST were found in all tissues when compared to other groups. A similar trend was observed in all groups after three weeks of exposure. The ALT levels were significantly higher (P<0.05) in lead + cadmium treated group , followed by cadmium > lead (Fig. 7 a - b).

According to the two way analysis of variance (two way ANOVA), from the present study, it was observed that lead + cadmium mixture has an increased effect on AST & ALT levels when compared to lead exposure or cadmium exposure. Besides, AST levels in all organs of week 3 were found significantly higher than week 1 (Fig. 6).

Table 2. Effect of lead and cadmium on superoxide dismutase (SOD) activity in different organs of the fish, *Oreochromis niloticus*

Groups	Superoxide dismutase (U/g protein)			
	Liver	Gills	Muscle	Kidney
		Week 1		
Control	$0.655 \pm 0.016^{a,A}$	$0.507 \pm 0.013^{a,A}$	$0.779 \pm 0.009^{a,A}$	$0.668 \pm 0.007^{a,A}$
Lead	$0.722 \pm 0.006^{a,B}$	$0.521 \pm 0.007^{a,B}$	$0.791 \pm 0.005^{a,B}$	$0.692 \pm 0.004^{b,B}$
Cadmium	$0.842 \pm 0.022^{b,C}$	$0.654 \pm 0.013^{b,C}$	$0.803 \pm 0.011^{a,C}$	$0.809 \pm 0.006^{c,C}$
Lead + Cadmium	$1.23 \pm 0.048^{c,D}$	$0.716 \pm 0.006^{c,D}$	$1.14 \pm 0.020^{b,D}$	$0.910 \pm 0.011^{d,D}$
		Week 3		
Control	$0.659 \pm 0.004^{a,NS,A}$	$0.494 \pm 0.004^{a,NS,A}$	$0.808 \pm 0.004^{a,NS,A}$	$0.698 \pm 0.004^{a,*,A}$
Lead	$0.708 \pm 0.005^{b,NS,B}$	$0.627 \pm 0.005b,*,B$	$0.883 \pm 0.004^{b,*,B}$	$0.776 \pm 0.017^{b,*,B}$
Cadmium	$0.948 \pm 0.016^{c,*,C}$	$0.739 \pm 0.009c,*,C$	$0.918 \pm 0.010^{b,*,C}$	$0.834 \pm 0.012^{c,*,C}$
Lead + Cadmium	$1.20 \pm 0.008^{d,NS,D}$	$0.804 \pm 0.001d,*,D$	$1.22 \pm 0.023^{c,*,D}$	$0.955 \pm 0.005^{d,*,D}$

Values are expressed as Mean ± SEM. Superscripts a,b,c,d in each column indicate significant difference(P<0.05) between groups in each week using one way ANOVA and Duncan's Multiple Range Test (DMRT) post hoc. Superscripts * and NS in each column indicate significant differences (P<0.05) and non significant difference between respective groups of week 1 and week 3 using Student t-test analysis. A , B, C and D superscripts indicate significant differences(P<0.05) between groups using two way ANOVA and Duncan's Multiple Range Test (DMRT) post hoc.

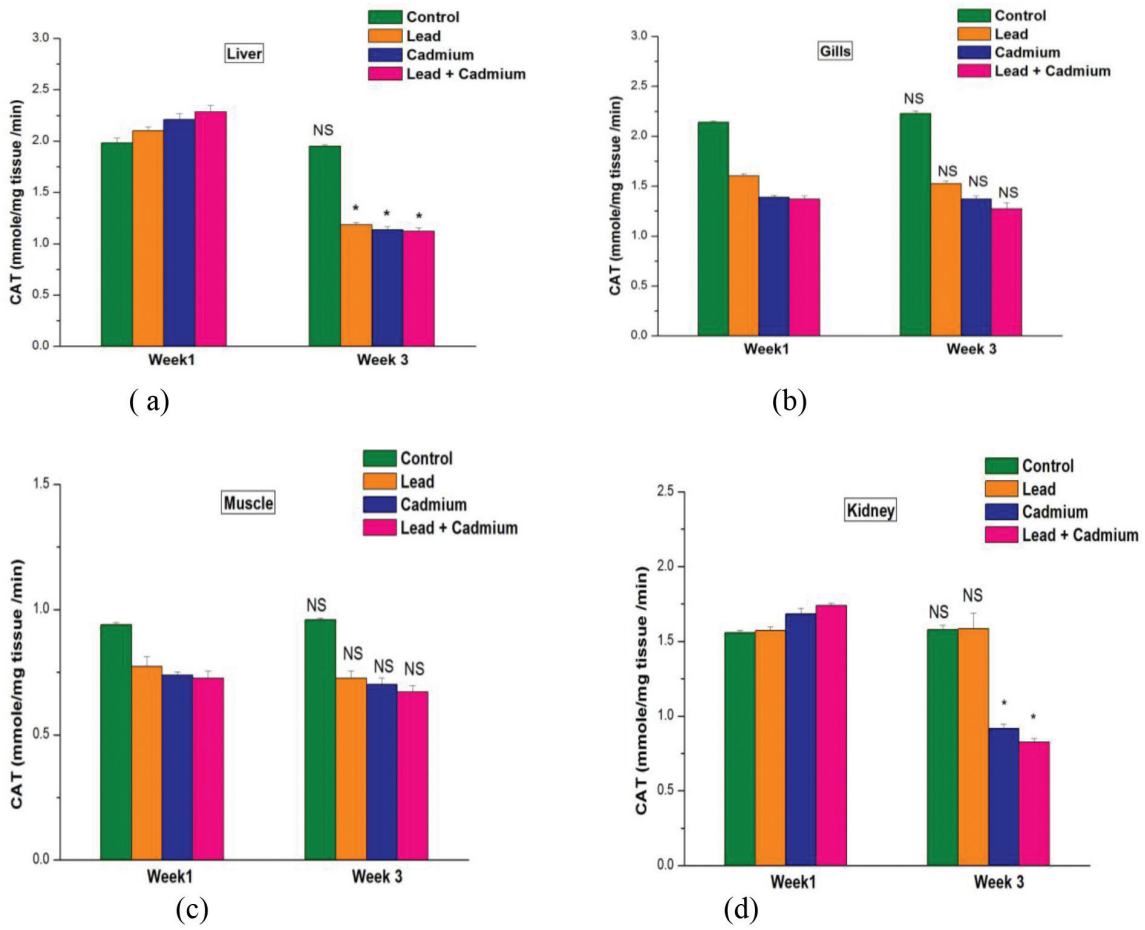

Fig. 2. Effect of lead and cadmium on catalase (CAT) activity in week 1 and week 3 of the fish, Oreochromis niloticus

Catalase (CAT) levels in (a) liver, (b) gills (c) muscle and (d) kidney of fish, *Oreochromis niloticus*. Values are Mean ± SEM of six animals. Different superscripts (*) indicate significant differences (P<0.05) and NS indicates non - significant difference between week 1 and week 3 of each group using Student t-test analysis.

DISCUSSION

Changes in antioxidants

Oxidative stress in aquatic organisms may lead to ROS (Reactive oxygen species) production and alterations in antioxidant enzymes (Livingstone, 2001). CAT and SOD are part of the cellular enzymatic antioxidant defense systems and they act

Table 3. Effect of lead and cadmium on aspartate aminotransferase (AST) activity in different organs of the fish, *Oreochromis niloticus*

Groups	AST (Aspartate aminotransferase) (μm/g)			
	Liver	Gills	Muscle	Kidney
		Week 1		
Control	459.83 ± 2.74^{a,A}	251.16 ± 3.40^{a,A}	264.50 ± 2.34^{a,A}	282.33 ± 0.98^{a,A}
Lead	465.83 ± 12.13^{a,B}	272.16 ±3.30^{b,B}	279.16 ± 2.67^{b,B}	291.66 ± 8.40^{ab,B}
Cadmium	502.00 ± 6.53^{b,C}	280.33 ± 2.44^{b,C}	281.50 ± 1.84^{b,C}	301.66 ± 1.17^{bc,C}
Lead + Cadmium	516.60 ± 7.72^{b,D}	282.16 ± 5.67^{b,D}	284.83 ± 1.90^{b,D}	314.33 ± 1.52^{c,D}
		Week 3		
Control	454.66 ± 6.38^{a,NS,A}	244.50± 2.62^{a,NS,A}	267.16 ± 3.53^{a,NS,A}	276.16 ± 1.75^{a,NS,A}
Lead	482.66 ± 4.46^{b,NS,B}	272.16 ± 1.45^{b,NS,B}	282.00 ± 5.13^{b,NS,B}	301.33 ± 1.05^{b,NS,B}
Cadmium	545.33 ± 9.79^{c,*,C}	281.50 ± 2.59^{c,NS,C}	282.83 ± 2.70^{b,NS,C}	304.16 ± 0.70^{b,NS,C}
Lead + Cadmium	546.50 ± 4.48^{c,*,D}	283.50 ± 4.47^{c,NS,D}	285.50 ± 2.60^{b,NS,D}	316.83 ± 3.11^{c,NS,D}

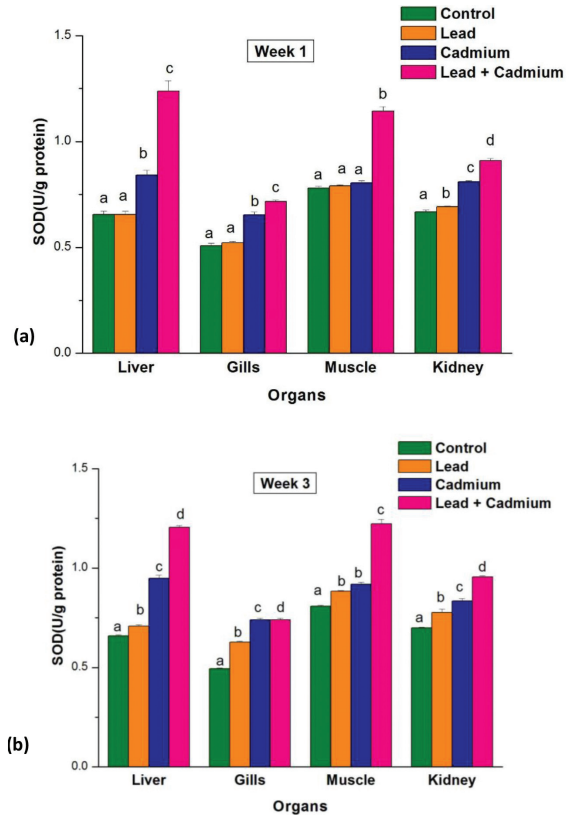

Fig. 3. Effect of lead and cadmium on superoxide dismutase (SOD) activity in different organs of the fish, *Oreochromis niloticus*

Superoxide dismutase (SOD) levels in (a) week 1 and (b) week 3 in liver, gills, muscle and kidney of the fish, *Oreochromis niloticus*. Values are Mean ± SEM of six animals. Different superscripts a, b, c indicate significant differences (P<0.05) among different groups using One way ANOVA and DMRT post hoc.

in combination to counteract oxidative stress (Waalkes, 2000; Zhang *et al*., 2009; Feng *et al*., 2013; Zheng *et al*., 2013). The variation in the activity of antioxidant enzymes may be used as indicators of oxidative stress due to pollutants (Sayeed *et al*., 2003). SOD catalyzes the breakdown of superoxide radicals to hydrogen peroxide. CAT prevents oxidative stress by degrading hydrogen peroxide (Wang *et al*., 2011).

In the present study, catalase activity showed significant changes in all the tissues (Table 1 and Fig. 1&2). After one week of exposure, an increase in catalase activity was noted in liver and kidney in all the groups with highest activity in lead + cadmium treated group followed by cadmium > lead. However a decrease in its activity was noted in the gills and muscle in the order lead + cadmium mixture > cadmium > lead. Catalase activity decreased significantly (P< 0.05) in all the tissues at the end of three weeks of exposure. Similar results of decrease in catalase activity have been reported in liver and kidney by Dabas *et al*. (2011) in *Labeo rohita*, Naveed *et al*.(2010) in *Channa punctatus* exposed to triazophos for 24,48,72 and 96 hrs, Chitra and Maiby (2014) in *Oreochromis mossambicus* exposed to sublethal concentration of bisphenol A (BPA) and Faheem *et al*. (2012) in *Oreochromis niloticus* upon exposure to cadmium. A reduction in catalase activity in the gills has been reported by Abhijith *et al*.(2016) in fish exposed to Methyl Parathion (MP) throughout the 35 days study period, Chitra and

Table 4. Effect of lead and cadmium on alanine aminotransferase (ALT) activityin different organs of the fish, *Oreochromis niloticus*

Groups	ALT (Alanine aminotransferase) (nmole/min/mg)			
	Liver	Gills	Muscle	Kidney
		Week 1		
Control	$0.508 \pm 0.011^{a, A}$	$0.330 \pm 0.008^{a,A}$	$0.442 \pm 0.008^{a,A}$	$0.240 \pm 0.014^{a,A}$
Lead	$0.633 \pm 0.011^{b,A}$	$0.412 \pm 0.022^{b,A}$	$0.492 \pm 0.015^{b,A}$	$0.302 \pm 0.011^{b,A}$
Cadmium	$1.03 \pm 0.063^{c,B}$	$0.462 \pm 0.031^{b,B}$	$0.542 \pm 0.011^{c,B}$	$0.417 \pm 0.014^{c,B}$
Lead + Cadmium	$1.42 \pm 0.026^{d,C}$	$0.645 \pm 0.015^{c,C}$	$0.648 \pm 0.020^{d,C}$	$0.530 \pm 0.013^{d,C}$
		Week 3		
Control	$0.512 \pm 0.012^{a,NS,A}$	$0.340 \pm 0.007^{a,NS,A}$	$0.446 \pm 0.009^{a,NS,A}$	$0.255 \pm 0.021^{a,NS,A}$
Lead	$0.680 \pm 0.017^{b,NS,A}$	$0.438 \pm 0.011^{b,NS,A}$	$0.507 \pm 0.007^{b,NS,A}$	$0.353 \pm 0.0194^{b,NS,A}$
Cadmium	$1.33 \pm 0.086^{c,*,B}$	$0.480 \pm 0.022^{c,NS,B}$	$0.583 \pm 0.016^{c,NS,B}$	$0.433 \pm 0.018^{c,NS,B}$
Lead + Cadmium	$1.53 \pm 0.015^{d,NS,C}$	$0.672 \pm 0.009^{d,NS,C}$	$0.702 \pm 0.021^{d,NS,C}$	$0.575 \pm 0.013^{d,NS,C}$

Values are expressed as Mean ± SEM. Superscripts a,b,c,d in each column indicate significant difference(P<0.05) between groups in each week using one way ANOVA and Duncan's Multiple Range Test (DMRT) post hoc. Superscripts * and NS in each column indicate significant differences (P<0.05) and non significant difference between respective groups of week 1 and week 3 using Student t-test analysis. A, B, C and D superscripts indicate significant differences (P<0.05) between groups using two way ANOVA and Duncan's Multiple Range Test (DMRT) post hoc.

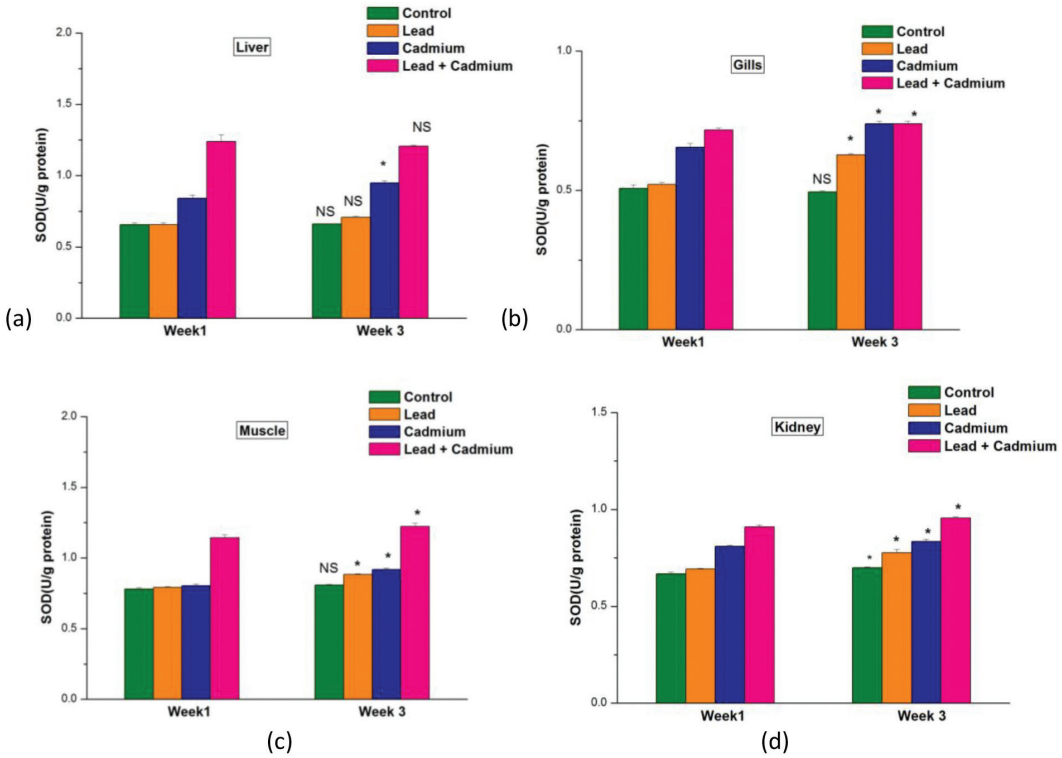

Fig. 4. Effect of lead and cadmium on superoxide dismutase (SOD) activity in week 1 and week 3 in the fish, *Oreochromis niloticus*

Superoxide dismutase (SOD) levels in (a) liver, (b) gills (c) muscle and (d) kidney of fish, *Oreochromis niloticus*. Values are Mean ± SEM of six animals. Different superscripts (*) indicate significant differences (P<0.05) and NS indicates non - significant difference between week 1 and week 3 of each group using Student t-test analysis.

Sajhita (2014) in *Oreochromis mossambicus* exposed to 1 ppm BPA for 10 and 20 days. Vutukuru *et al*. (2006) reported a significant decrease in catalase activity in gills due to inactivation by the superoxide radical triggered by MP exposure. A decrease in catalase activity was also reported by Li *et al*.(2016) in all vital organs of Japanese medaka exposed to 1.5 mg/l BPA. Wu *et al*. (2011) noted a significantly reduced catalase activity in zebrafish exposed to graded concentration (0.1-1000 µg/L-). Dosages and durations of heavy metal exposure can have adverse effects on the activity of some antioxidant enzymes (Chandran *et al*., 2005; Zhang *et al*., 2009; Cong *et al*., 2012). The reduction of the catalase enzyme activity may be due to direct metal mediated structural alteration of the enzyme (Arillo *et al*., 1982), depression of catalase synthesis (Pruell and Engelhardt, 1980), inhibition of the enzyme activity by superoxide radicals (Kono and Fridovich, 1982) or inactivation of enzyme by overproduction of ROS (Pigeolet *et al*., 1990). In the present study it is observed that lead + cadmium mixture has a greater effect on catalase activity in all the organs compared to single application of these metals.

The SOD activity increased significantly (P<0.05) at the end of three weeks of exposure in all the tissues compared to first week (Table 2 and Fig. 3&4). The activity was found to be highest in the liver compared to other tissues followed by muscle and kidney and also the highest increase was observed in the group exposed to lead + cadmium mixture. Superoxide anions might have been produced in response to these metals leading to elevation in SOD levels which helps in converting them to less harmful hydrogen peroxide (H_2O_2). Time-dependent increase in SOD activity in tissues observed in the present study may be an indicator of compensatory tissue response to convert the superoxide radical to H_2O_2 induced by metal exposure. Similar results were obtained by Dabas *et al*. (2011) in *Labeo rohita* exposed to cadmium. Soares *et al*. (2008) and Osman *et al*. (2009) worked on cadmium induced oxidative stress in various fish species and reported an increase in SOD activity in liver and kidney. Contrary to the present finding

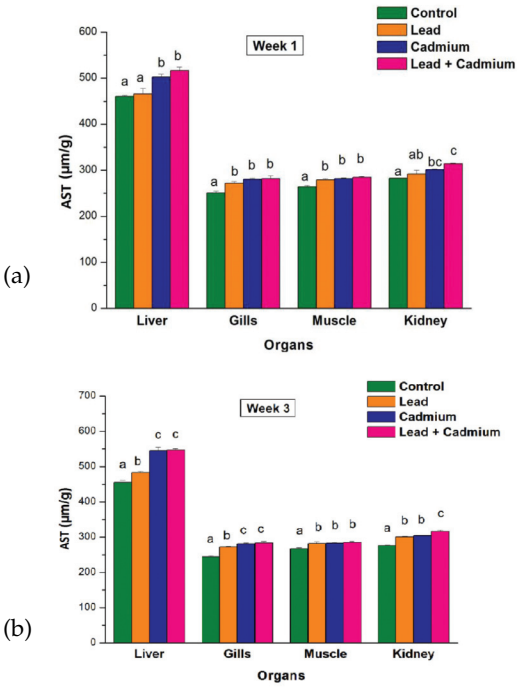

Fig. 5. Effect of lead and cadmium on aspartate aminotransferase (AST) levels in different organs of the fish, *Oreochromis niloticus*

(b) Aspartate aminotransferase (AST) levels in (a) week 1 and (b) week 3 on liver, gills, muscle and kidney of fish, *Oreochromis niloticus*. Values are Mean ± SEM of six animals. Different superscripts a, b, c indicate significant difference (P<0.05) among different groups using One way ANOVA and DMRT post hoc.

Garcia Sampaio *et al*. (2008) showed a significant decrease in SOD level in the fish, *Piaractus mesopotamicus* after exposure to copper. This they suggested might be an indicator of damage in the antioxidant mechanism.

While comparing the effect of metals on enzyme activity, it is observed that lead + cadmium (mixture) treated group has a greater impact on SOD activity in all organs compared to cadmium or lead applied singly. However not much work has been done to compare the effect of metal mixture in aquatic organisms with that of exposure to metals singly. Vanegas *et al*. (1997) reported great increase in toxicity of metals when both were present in mixtures, as compared to their presence individually. They found that at similar exposure times, cadmium in combination with zinc increased in toxicity 4 to 6 times.

Changes in AST and ALT

In different studies various responses of AST and ALT activities were recorded depending upon the metal species, its concentration and duration of exposure (Zikic *et al*., 2001; Vutukuru *et al*., 2007). In the present study an increase in the AST and ALT was observed in all the groups compared to control (Table 3 & 4 and Fig. 5 to 8). Similar results were obtained by Das *et al*. (2004) in Indian major carps exposed to nitrite toxicity and they suggested that

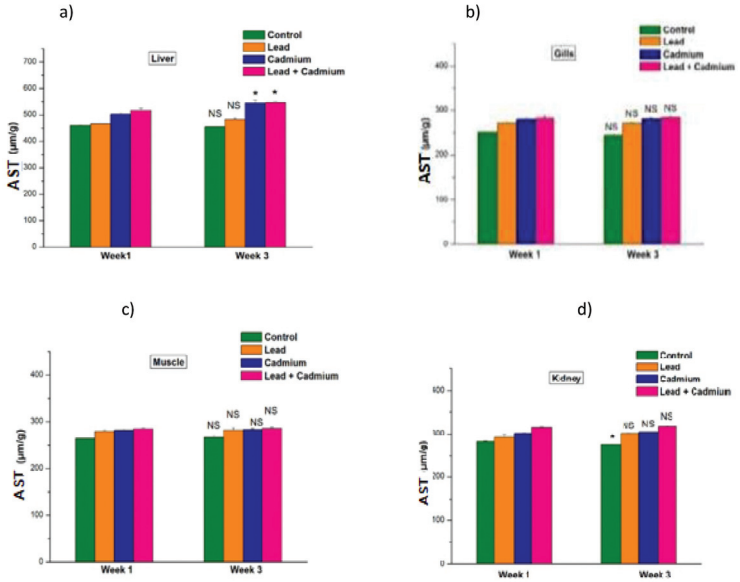

Fig. 6. Effect of lead and cadmium on aspartate aminotransferase (AST) levels in week 1 and week 3 of the fish, *Oreochromis niloticus*

Aspartate aminotransferase (AST) levels in (a) liver, (b) gills (c) muscle and (d) kidney of fish, *Oreochromis niloticus.* Values are Mean ± SEM of six animals. Different superscripts (*) indicate significant differences (P<0.05) and NS indicates non - significant difference between week 1 and week 3 of each group using Student t-test analysis.

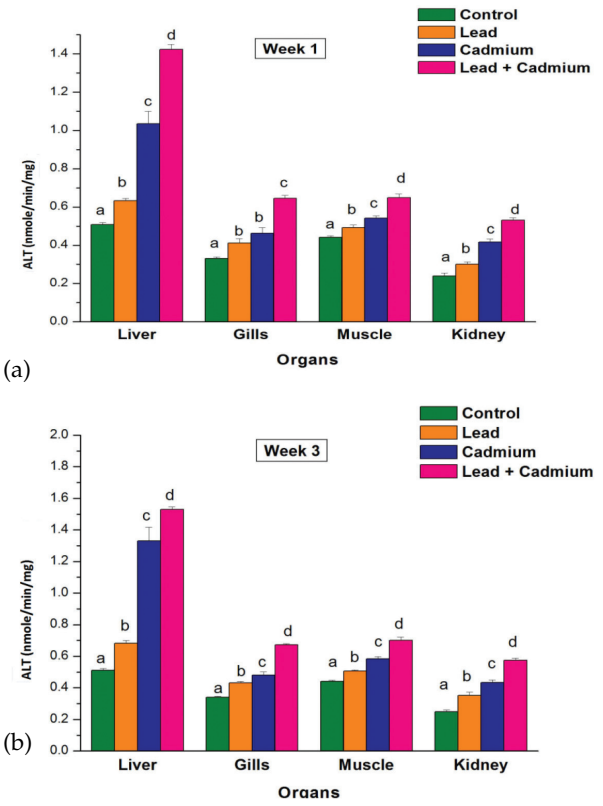

Fig. 7. Effect of lead and cadmium on alanine aminotransferase (ALT) levels in different organs of the fish, Oreochromis niloticus

Alanine aminotransferase (ALT) levels in (a) week 1 and (b) week 3 on liver, gills, muscle and kidney of fish, *Oreochromis niloticus*. Values are Mean ± SEM of six animals. Different superscripts a, b, c and d indicate significant difference (P<0.05) among different groups using one way ANOVA and DMRT post hoc.

the elevation of the transferases is a result of the diversion of the alphamino acids in the TCA cycle as keto acids to augument energy production. An increase in activities of AST and ALT in the muscle and liver of common carp exposed to 10 µg/L of cyfluthrin was observed by Sepici-Dincel *et al*. (2009).

De Smet and Blust (2001) also reported elevated activities of AST and ALT in liver, kidney and gills of *Cyprinu scarpio* following 0.8, 4.0 and 20 mg/l Cd exposures for 29 days which they predicted might be due to increased protein breakdown to deal with the energy requirement. Studies by Vutukuru *et al*. (2007) indicated that chromium caused a significant increase in the enzyme activities possibly due to leakage of enzymes across damaged plasma membranes or the increased synthesis of enzymes by the liver. Begum (2005), working at the sublethal concentrations of cypermethrin also observed an increase in AST and ALT in the liver, and gill tissues of *Clarias batrachus*. Yildirim *et al*. (2006) exposed *Oreochromis niloticus* to deltamithrin for four days and observed increase in enzyme activities (AST and ALT) in the gill, liver and kidney and assumed that the observed enzyme elevation is intended to increase the role of proteins in the energy production during toxicant stress.Usha and Raj (1993) have reported an increase in the level of AST and ALT in the animals exposed to vanadium. Mukhopadhyay *et al*.(1982) have stated that an increased transaminase activity in the liver of *Clarius batrachus* was due to the exposure to sublethal concentration of carbofuron and is compatible with liver damage. The enhancement of the aminotransferase activities may occur in order to counter the energy demand during metal stress. The activity of AST and ALT can be used to indicate the tissue damage of liver and kidney (Nemcsok and Boross, 1990). Thus, alterations in AST and ALT level can be used as a biomarker to assess the levels of contamination in the environment and toxicity of metals before the occurrence of detrimental effects .

Alanine aminotransferase (AST) is responsible for transferring amino group from alanine to 2-ketoglutaric acid forming glutamate and pyruvate. It is well known for tissue damage and its level rises higher in most types of hepato cellular damage (Tiwari and Srivastava, 2001).

In the present study lead + cadmium mixture induces the highest aminotransferase activity in all the organs compared to single application of the metals. Duration of exposure also has an impact on the enzyme activity. The study indicated that highest activity of AST and ALT was at the end of the third week of exposure compared to first week in all the groups as well as in all the organs . These results are in agreement with that of Shalaby (2007)who reported significant increase in AST and ALT of *O. niloticus* exposed to4.64 mg/L (¼ of 96 h LC50) of Cd after 15 and 45 days of exposure. Mekkawy *et al*. (2011) also obtained similar results for these enzymes in *O. niloticus* exposed to cadmium for 15 and 30 days. The significant increase of these enzymes in the tissues seems to indicate possible dysfunction taking place in the tissues of animals (Casilla *et al*., 1983). Usha and Raj (1993) have reported increase in the level of AST and ALT enzymes as a specific indication of hepatic cell damage and found them to be time and dose dependent.

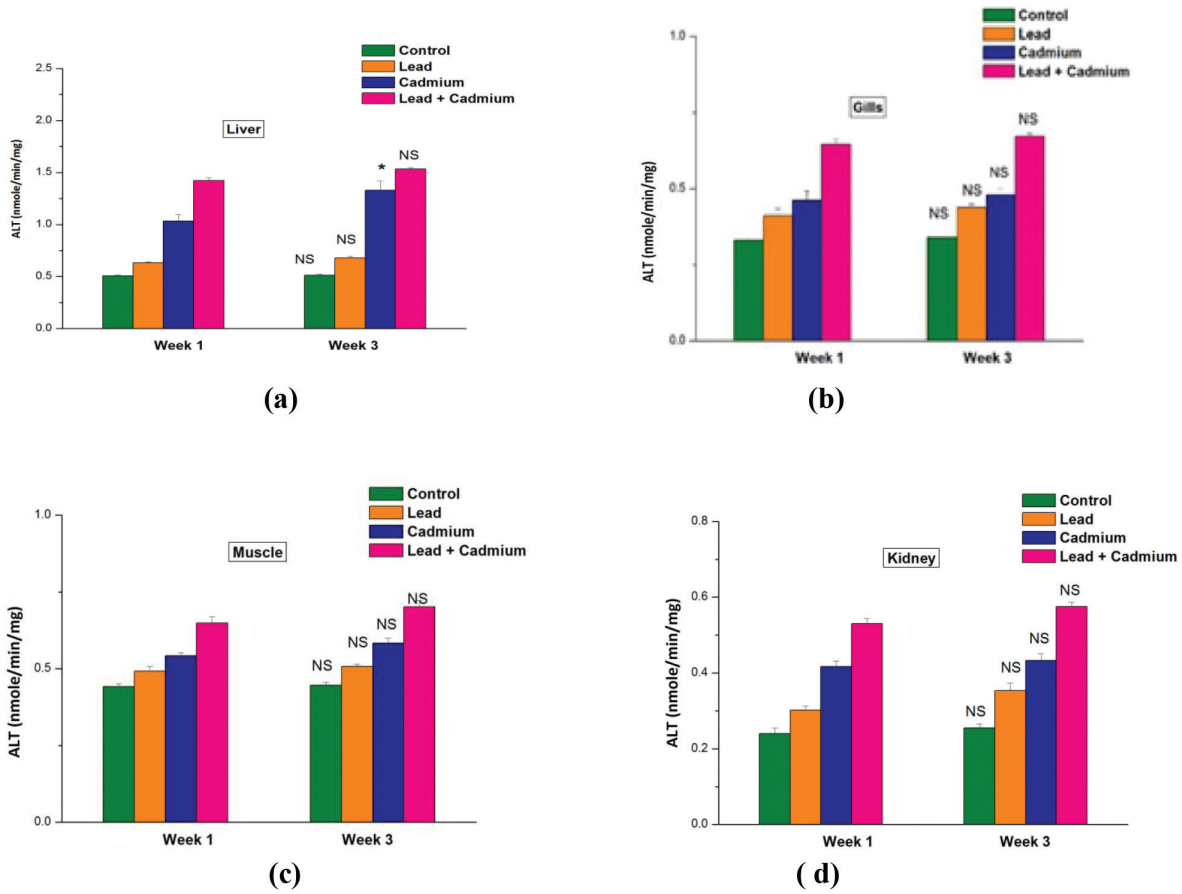

Fig. 8. Effect of lead and cadmium on alanine aminotransferase (ALT) levels in week 1 and week 3 of the fish, *Oreochromis niloticus*

Alanine aminotransferase (ALT) levels in (a) liver, (b) gills(c) muscle and (d) kidney of fish, *Oreochromis niloticus*. Values are Mean ± SEM of six animals. Different superscripts (*) indicate significant differences (P<0.05) and NS indicates non – significant difference between week 1 and week 3 of each group using Student t-test analysis.

CONCLUSION

From the present study it can be concluded that heavy metals cause oxidative stress in organisms and affects its metabolism. Changes in metabolic enzymes and antioxidants can be used as biomarkers to study the toxicity of heavy metals. Some metals when they come in combination are more toxic (synergistic effect) than when present individually. In the natural environment organisms are exposed to multiple pollutants, but most works are focussed on the effects of single metal exposure. In the present study lead and cadmium mixture exhibited a combined effect on the activity of enzymes and antioxidants in the fish. The impact of metal mixture was found to be more deleterious than its individual exposure. The present work indicates the need to study the impact of metal mixtures occurring in natural environment.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to the Director, School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala for all the support and facilities provided to carry out the research.

REFERENCES

Abhijith, B.D., Ramesh, M. and Poopal, R.K. 2016. Responses of metabolic and antioxidant enzymatic activities in gill, liver and plasma of *Catla catla* during methyl parathion exposure. *J. Basic Appl. Zool.* 77 : 3140. DOI: 10.1016/j.jobaz.2015.11.002.

Aebi, H. 1984. *Catalase in vitro. In: Packer L* (ed.) Methods in enzymology. *Academic Press Orlando* 105 : 121-126.

American Public Health Association. APHA., 2005. *American Water Works Association. AWWA.*

Standard methods for the examination of water and wastewater 2005. 21st edition. Washington, DC: American Public Health Association.

Arillo, A., Targiocco, C., Melodia, F. and Mensi, F. 1982. Biochemical effects of long-term exposure to Cr, Cd, Ni on rainbow trout (*Salmo gairdneri*) Rich.- influence of sex and season. *Chemosphere*. 11 : 47-57.

Begum, G. 2005. In vivo biochemical changes in liver and gill of *Clarias battrachus* during cypermethrin exposure and following cessation of exposure. *Pesticide and Biochemical Physiology*. 82 : 185 - 199.

Boulanger, E., Barst, B.D., Alloy, M.M., Blais, S., Houde, M. and Head, J. A. 2019. Assessment of environmentally contaminated sediment using a contact assay with early lifestage zebrafish (Danio rerio). *Sci. Total Environ.* 659 : 950-962.

Casilla, E., Meyers, M. and Ames, W. 1983. Relationship of serum chemistry values to liver and kidney histopathology in English sole (*Parophyrys vetalus*) after acute exposure to carbon tetrachloride. *Aquat. Toxicol*. 3 : 61-78.

Chandran, R., Sivakumar, A.A., Mohandass, S. and Aruchami, M. 2005. Effect of cadmium and zinc on antioxidant enzyme activity in the gastropod, *Achatina fulica*. *Comp Biochem Physiol. Part C*. 140: 422-426.

Chitra, K.C. and Sajitha, R. 2014.Effect of bisphenol-A on the antioxidant defense system and its impact on the activity of succinate dehydrogenase in the gill of freshwater fish, *Oreochromis mossambicus*. *J Cell Tissue Res*. 14 (2) : 4219-4226.

Chitra, K.C. and Maiby, S. 2014. Oxidative stress of Bisphenol-A and its adverse effects on liver of fresh water fish *Oreochromis mossambicus*. *IJSR*. 3(7) : 221-224.

Cicik, B. and Engin, K. 2005.The effect of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio*. *Turkian Journal of Vetnary Animal Sciences*. 31 : 113-117.

Cong, M., Wu, H.F., Liu, X.L., Zhao, J.M., Wang, X., Lv, J.S. and Hou, L. 2012. Effects of heavy metals on the expression of a zinc-inducible metallothionein-III gene and antioxidant enzyme activities in *Crassostrea gigas*. *Ecotoxicology*. 21 : 1928-1936.

Dabas, A., Nagpure, N. S., Mishra, R. M. Kumar, R., Kushwaha, B. and Srivastava, R. 2011. Assessment of cadmium induced alteration in superoxide dismutase and catalase activities of *Labeo rohita* (Hamilton, 1822). *J. Ecophysiol. Occup. Hlth*. 11 : 205-211.

Das, P.C., Ayyappan, S., Das, B.K. and Jena, J.K. 2004. Nitrite toxicity in Indian major carps: sublethal effect on selected enzymes in fingerlings of *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*. *Comparative Biochemistry and Physiology*. 138 : 3-10.

Dewanjee, S., Sahu, R., Karmakar, S. and Gangopadhyay, M. 2013. Toxic effects of lead exposure in Wistar rats: involvement of oxidative stress and the beneficial role of edible jute (*Corchorus olitorius*) leaves. *Food Chem. Toxicol*. 55: 78–91. https://doi.org/10.1016/j. fct.2012.12.040.

Ercal, N., Gurer-Orhan, H.and Aykin-Burns, N. 2001. Toxic metals and oxidative stress Part I: Mechanisms involved in metal-induced oxidative damage. *Curr. Top. Med. Chem*. 1(6): 529-539. https://doi.org/10.2174/1568026013394831.

Faheem, M., Sulehria, A.Q.K., Tariq, M., Khadija, I. and Saeed, M. 2012. Effect of sub-lethal dose of cadmium chloride on biochemical profile and catalase activity in fresh water fish *Oreochromis niloticus*. *Biologia* (Pakistan). 58(1-2) : 73-78.

Fang, W., Peng, Y., Muir, D., Lin, J. and Zhang, X. 2019. A critical review of synthetic chemicals in surface waters of the US, the EU and China. *Environ. Int.* 131 : 104994.

Feng, M.B., Li, Y., Qu, R.J., Wang, L.S. and Wang, Z.Y. 2013. Oxidative stress biomarkers in freshwater fish *Carassius auratus* exposed to decabromodiphenyl ether and ethane, or their mixture. *Ecotoxicology*. 22: 1101–1110.

Feo, M.L., Ginebreda, A., Eljarrat, E. and Barceló, D. 2010. Presence of pyrethroid pesticides in water and sediments of Ebro River Delta. *J. Hydrol.* 393 : 156–162.

Finney, D.J. 1971. *Probit Analysis*. Cambridge: Cambridge University Press : 333 p.

Garcia Sampaio, F.G., Boijink, C.L., Oba, E.T., Santos, L.R.B., Kalinin, A.L. and Rantin, F.T.2008. Antioxidant defenses and biochemical changes in pacu *Piaractus mesopotamicus* in response to single and combined copper and hypoxia exposure. *Comparative Biochemistry and Physiology*. 147 : 43-51. doi:10.1016/j.cbpc.2012.07.002.

Geoffroy, L., Frankart, C. and Eullaffroy, P. 2004. Comparison of different physiological parameter responses in Lemna minor and *Scenedesmus obliquus* exposed to herbicide flumioxazin. *Environ. Pollut*. 131 : 233-241.

Gravato, C., Teles, M., Oliveira, M. and Santos, M.A. 2006. Oxidative stress, liver biotransformation and genotoxic effects induced by copper in *Anguilla anguilla* L. - the influence of pre-exposure to bnaphthoflavone. *Chemosphere*. 65 : 1821–1830. doi:10.1016/ j.chemosphere.2006.04.005.

Heath, A.G. 1995. *Water Pollution and Fish Physiology*. CRC Press, Florida, USA, 245.

Heath, A.G. 1987. Effects of waterborne copper or zinc on the osmorregulatory response of bluegill to a hypertonic Na Cl challenge. *Comp Biochem Physiol*. 88C : 307-311.

Kakkar, P., Das, B. and Viswanathan, D.N.1984 .A

modified spectrometric assay of superoxide dismutase. Ind. *J Biochem. Biophys*. 21 : 130.

Kono, Y. and Fridovich, I. 1982. Superoxide radical inhibits catalase. *J. Biol. Chem*. 257 : 5751-5754.

Lao, W., Tsukada, D., Greenstein, D.J., Bay, S.M. and Maruya, K.A. 2010. Analysis, occurrence, and toxic potential of pyrethroids, and fipronilin sediments from an urban estuary. *Environ. Toxicol. Chem*. 29: 843–851.

Levesque, H.M., Moon, T.W., Campbell, P.G.C. and Hontela, A. 2002. Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Perca flavescens*) chronically exposed to metals in the field. *Aquat Toxicol*. 60 : 257-267. doi:10.1016/S0166445X(02)00012-7.

Li, D., Chenb, Q., Caoc, J., Chena, Li, H. L., Cedergreend, N., Xiee, H. and Xiea, L. 2016. The chronic effects of lignin-derived bisphenol and bisphenol A in Japanese medaka *Oryzia slatipes*. *Aquatic Toxicology*. 170 : 199- 207.

Livingstone, D.R. 2001. Contaminant-stimulated reactive oxygenspecies production and oxidative damage in aquatic organisms. *Mar. Pollut. Bull*. 42 : 656-666.

Malarvizhi, A., Kavitha, C., Saravanan, M. and Ramesh, M. 2012. Carbamazepine (CBZ) induced enzymatic stress in gill, liver and muscle of a common carp, *Cyprinus carpio*. *J. King Saud Univ*. 24 : 179-186.

Malik, N., Marwan, N. and Kurths, J. 2010. Spatial structures and directionalities in monsoonal precipitation over south Asia. Nonlinear Process. *Geophys.* 17 : 371-381.

Mekkawy, A.A., Mahmoud, U.M., Wassif, E.T. and Naguib, M. 2011. Effects of cadmium on some haematological and biochemical characteristics of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. *Fish Physiol. Biochem*. 37 : 71-84.

Meyer, A.P., Brown, M.J. and Falk, H. 2008. Global approach to reducing lead exposure and poisoning. *Mutat. Res*. 659(1-2): 166-175. https://doi.org/10.1016/j.mrrev.2008.03.003.

Mukhopadhyay, P.K., Mukherjee, A.P. and Dehadrai, P.V. 1982. Certain biochemical responses in the air breathing catfish *Clarias batrachus* exposed to sublethal carbofuran. *Toxicology*. 23 : 337-345.

Nabinger, D.D., Altenhofen, S., Bitencourt, P.E.R., Nery, L.R., Leite, C.E. and Vianna, M.R. 2018. Nickel exposure altersbe havioralparametersinlar valandadultze brafish. *Sci. Total Environ.* 624 : 1623–1633.

Naveed, A., Janaiah, C. and Venkateshwarlu, P. 2010. The effects of lihocin toxicity on Protein metabolism of the freshwater edible fish, *Channa punctatus* Bloch. *J Toxicol. Environ. Health Sci*. 3(1) : 18-23.

Nemcsok, J. and Boross, L. 1982. Comparative studies on the sensitivity of different fish species to metal pollution. *Acta Biol.* 33 : 23-27.

Nemcsok, J. and Boross, L. 1990. Effect of sublethal concentration of phenol on some enzyme activities and blood sugar level of carp Cyprinuscarpio. *Environ. Monit Assess*. 14: 377-383.

Osman, A.G.M., Reheem, A.E.B.M.A.E., Abuel Fadl, K.Y., and Gad El Rab, A.G. 2010. Enzymatic and histopathologic biomarkers as indicators of aquatic pollution in fishes. *Nat. Sci*. 2 (11): 1302-1311.

Osman, H. A. M., Ibrahim, T. B., Ali, A. T. and Derwa, H. I. M. 2009. Field Application of Humic Acid Against the Effect of Cadmium Pollution on Cultured Tilapia *Oreochromis niloticus*. *World Appl. Sci. J*.6: 1569-1575.

Pande, M. and Flora, S.J. 2002. Lead induced oxidative damage and its response to combined administration of alpha-lipoic acid and succimers in rats. *Toxicol*. 177(2-3): 187–196. https://doi. org/10.1016/S0300-483X(02)00223-8.

Patrick, L. 2006. Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. *Altern. Med. Rev*. 11(2): 114-127.

Pigeolet E., Corbisier, P., Houbion, A., Lambert, D., Michiels,, C., Raes,, M., Zachary, M.D. and Remacle, J. 1990. Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. *Mechanisms of Ageing and Development*. 51 : 283–297.

Pruell, R. J. and Engelhardt, F. R. 1980. Liver cadmium uptake, catalase inhibition and cadmium thionein production in the killifish (*Fundulus heteroclitus*) induced by experimental cadmium exposure. *Mar. Environ. Res*. 3 : 101-111.

Reitman, S. and Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol*. 28 : 56.

Sary, A. A. and Beheshti, M. 2011. Cadmium, Iron, Lead and Mercury Bioaccumulation in Abu Mullet, Liza abu in different tissues from Karoun and Karkheh Rivers, Khozestan, *Iran.Bulletin of Environmental Contamination and Toxicology.* 88 (2) : 158-61.DOI: 10.1007/s00128-011-0476-8.

Sayeed, I., Parvez, S., Pandey, S., Bin-Hafeez, B., Haquee, R. and Raisuddin, S. 2003. Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus* Bloch. *Ecotoxicol. Environ. Saf*. 56 : 295-301.

Sepici-Dincel, A., Karasu, B.C., Selvi, M., Sarikaya R., Sahih, D., Ozkul, I.A. and Erkve, F. 2009. Sublethal cyfluthrin to carp *Cyprinus carpio* fingerlings: Biochemical, hematological, histopathologic alalterations. *Ecotoxicology and Environmental Safety*. 72 : 1433-1439.

Shalaby, A.M.E. 2007. Effect of EDTA on toxicity reduction ofcadmium in relation to growth, some

haematological and bio-chemical profiles of Nile tilapia (*Oreochromis niloticus*). *J. Fish. Aquatic Sci.* 2 (2) : 100-109.

Soares, S. S., Martins, H., Gutiérrez-Merino, C. and Aureliano, M. 2008. Vanadium and cadmium in vivo effects in teleost cardiac muscle: Metal accumulation and oxidative stress markers. *Comparative Biochemistry and Physiology*, Part C. 147 : 168-178.

Sprague, J.B. 1971. Measurement of pollution toxicity to fish, sub lethal effects and safe concentration. *Water Res*. 5 : 245-266.

Tiwari, A. and Srivastava, V.K. 2001. Acetaminophen poisoning induced enzymological and biochemical changes in liver and kidney of male mice. *Mus musculus*. *Biochem. Cell. Arch*. 1 : 71-76.

Turgut, C. 2003. The contamination with organochlorine pesticides and heavy metals in surface water in Küçük Menderes Riverin Turkey, 2000-2002. *Environ. Int.* 29 : 29-32.

UNEP. 1985. *GESAMP: Cadmium, lead and tin in the marine environmental*. Regional Seas Reports and Studies No. 56. UNEP

US EPA .2009. *National recommended water quality criteria. Office of Water, Office of Science and Technology (4304T).* http:// www.rsc.org/dose/title of subordinate document. Accessed 24 July 2013.

Usha, K. and Raj, V. 1993. Changes in the plasma level of phosphates and transaminases in rabbits following vanadium exposure. *Poll. Res*. 12 : 19-27.

Van der Oost, R., Beyer, J. and Vermeulen, N.P.E. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol*. 13 : 57-149.

Vanegas, C., Espina, S.,Botello, A.V., and Villanueva, S. 1997. Acute Toxicity and Synergism of Cadmium and Zinc in White Shrimp, *Penaeussetiferus,* Juveniles. *Bull. Environ. Contam. Toxicol.* 58 : 87-92.

Vutukuru, S.S., Chintada, S., Madhavi, K.R., Rao, J.V. and Anjaneyulu, Y. 2006. Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the freshwater teleost fish, *Esomus danricus*. *Fish Physiol. Biochem*. 32 (3) : 221-229.

Vutukuru, S.S., Prabhath, N.A., Raghavender, M. and Yerramilli, A. 2007. Effect of arsenic and chromium on the serum amino-transferases activity in Indian major carp, *Labeo rohita*. *Int J Environ Res Public Health*. 4 : 224-227.

Waalkes, M.P. 2000. Cadmium carcinogenesis in review. *J. Inorg Biochem*. 79 : 241-244.

Wang, L., Xu, T., Lei, W.W., Liu, D.M., Li, Y.J., Xuan, R.J. and Ma, J.J. 2011. Cadmium-induced oxidative stress and apoptotic changes in the testis of freshwater crab, *Sinopotamon henanense. PLos One*. 6(11) : 27853.

Webb, D., Gagnon, M.M. and Rose, T. 2005. Metabolic enzyme activities in black bream, *Acanthopagrus butcheri* from the swan canning estuary, Western Australia. *Comp. Biochem. Physiol*. C. 141: 356-365.

Wu, M., Xu, H., Shen, Y., Qiu, W. and Yang, M. 2011. Oxidative stress in zebrafish embryos induced by short-term exposure to bisphenol-A, nonylphenol, and their mixture. *Environ Toxicol Chem*. 30(10) : 2335-2341.

Yildirim, M.Z., Benli, K.C., Selvi, M., Ozkul, A., Erko, F. and Kocak, O. 2006. Acute toxicity behavioural changes and histopathological effects of deltamethrin on tissues (gills, liver, brain, spleen, kidney, muscle, skin) of Nile Tilapia (*Oreochromis niloticus*) fingerlings. *Environmental Toxicology*. 21: 614-620.

Zhang, Q.S., Hou, J.J., Liu, X.J., Luo, J.X. and Xiong, B.X. 2009. Effects of copper on antioxidant enzyme activities and metallothionein concentration of *Bellamya purificata*. *Acta. Hydrobiol. Sin.* 33(4) : 717-725.

Zhang, Q.S., Hou, J.J., Liu, X.J., Luo, J.X., Xiong, B.X., 2009. Effects of copper on antioxidant enzyme activities and metallothionein concentration of *Bellamya purificata*. *Acta. Hydrobiol. Sin*. 33(4): 717-725.

Zheng, S.M., Wang, Y.Y., Zhou, Q.X. and Chen, C.H. 2013. Responses of oxidative stress biomarkers and DNA damage on a freshwater snail (*Bellamya aeruginosa*) stressed by ethylbenzene. *Arch Environ Contam Toxicol*. 65 : 251-259.

Zheng, J.L., Yuan, S.S., Wu, C.W. and Lv, Z. M. 2016b. Acute exposure to waterborne cad-mium induced oxidative stress and immunotoxicity in the brain, ovary and liver of zebrafish (*Danio rerio*). *Aquat. Toxicol.* 180 : 36-44.

Zikic, R.V., Stajn, S., Pavlovic, Z., Ognjanovic, B.I. and Saicic, Z.S. 2001. Activities of superoxide dismutase and catalase in erytrocyte and plasma transaminases of goldfish (*Carassius auratus gibelio* Bloch.) exposed to cadmium. *Physiol Res*. 50 : 105-111.