Chemical Speciation & Bioavailability

ISSN: (Print) 0954-2299 (Online) Journal homepage: http://www.tandfonline.com/loi/tcsb20

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To cite this article: Anwar Ahmad & Daoud Alib (2013) Effect of battery-manufacturing effluent on endogenous antioxidant in freshwater fish, Chemical Speciation & Bioavailability, 25:2, 106-112

To link to this article: http://dx.doi.org/10.3184/095422913X13693327881996

Effect of battery-manufacturing effluent on endogenous antioxidant in freshwater fish

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ABSTRACT

This study monitors and assesses the effect of battery-manufacturing effluent containing metals Pb, Zn and Cd on endogenous antioxidants. Malonaldehyde (MDA), reduced glutathione sulfyhydryl (GSH) and catalase (CAT) which are known biomarkers of effluent were exposed to 0%, 25%, 50%, 75% and 90% amendments for 74h on the gills, liver and kidneys of *C. punctata*. There was more metal Zn accumulation in the gills and GSH contents increased significantly in the gills (P<0.01), liver accumulation of Pb was found to be more (P<0.05), whereas lowest accumulation of Pb was found in kidneys and the highest accumulation of Cd (P<0.05). Over all amendments of the effluents, MDA contents were increased in the gills, liver and kidneys (P<0.01). GSH levels were decreased among the liver and kidneys compared to the gills (P<0.01) at 90% amendment. Effluent exposure caused a significant decrease in the activities of CAT in the gills, liver and kidneys (P<0.01, 0.05 and 0.05) of fish. Increased MDA activity was indicative of the formation of free radicals in the fish with exposure to amendments of battery manufacturing effluent, while increased levels of GSH pointed to the occurrence of a scavenging mechanism of free radicals.

Keywords: battery manufacturing effluent, heavy metals, glutathione sulfyhydryl, malonaldehyde catalase, biomarker

INTRODUCTION

The unrestricted developmental activities (such as industrialisation and urbanisation) during the past few years in Sungai Lambing, Malaysia have given rise to serious problems of environmental contamination by battery-manufacturing industries. Metals are known to inhibit biochemical and physiological mechanisms vital for fish metabolism (Basha and Rani, 2003). In contaminated environments, organisms are exposed to mixtures of pollutants whose synergistic/ antagonistic effects are difficult to interpret and predict from chemical analysis alone; some contaminants strongly accumulate in tissues without inducing toxic effects, while others are characterised by elevated toxicity at low levels of exposure (Chen *et al.,* 2001; Perez *et al.,* 2001; Wanzenbock *et al.,* 2004; Fasulo *et al.,* 2008). Lead, zinc and cadmium have been known to be toxic to biota for decades. In a number of studies by Borgman *et al.,*(2005), lead, zinc and cadmium have been recognised as among the most toxic environmental and industrial pollutants due to their ability to induce severe alterations in various organs and tissues following either acute or chronic exposure (Gerhardt *et al.,* 2005; Franco-Ura *et al.,* 2010). Consequently, extensive studies have been carried out to identify the mechanisms of Pb, Zn and Cd toxicity and accumulation by fresh water invertebrats and fish (Ahmad and Alam, 2002, 2003; Canli *et al.,* 2001; Chen *et al.,* 2001; Yuanyuan *et al.,* 2006; Yadav and Trivedi, 2009).

Freshwater fish are a suitable target to measure the toxicity of metals, alteration in the levels of biomolecules and fish bioaccumulation of metals can be used as biomarkers in environmental risk assessment (Oost *et al.,* 3003). Other research has given a conceptual framework for using mussels as a biomonitor in effluent toxicity (Smolders *et al.,* 2003). Thus, during the past two decades, the use of biological responses (biomarkers) on particular test species has become relevant in toxicological assessments since it allows the early detection of overall effects of toxicity, providing information, even at the sub-lethal level (Canli *et al.,* 2001; Wall *et al.,* 2001; Santos *et al.,* 2004; Iqbal *et al.,* 2006; Schmitt *et al.,* 2007), and fish have been employed as sensitive indicators of their genotoxic and mutagenic effects (Yadav and Trivedi, 2009).

Doi: 10.3184/095422913X13693327881996

Heavy metals in the water are accumulated by the fish which affects the metallothionein acting as a biomarker of contamination (Papetti and Rossi, 2009). Most of the heavy metals create physiological stress leading to the generation of free radicals when in high concentration. Lead and cadmium, in turn, induce the production of reactive oxygen species (ROS) (Fatima *et al.,* 2000; Livingstone, 2001; Ruelas *et al.,* 2009; Ferreira *et al.,* 2010). Therefore, a mechanism to interrupt such an autocatalytic process is required. Under normal circumstances, concentration of oxygen radicals remains low because of the activity of protective enzymes, catalase (CAT) and glutathione sulfyhydryl (GSH), other conditions may enhance the protective processes such as the accumulation of compatible solutes and an increase in the activities of detoxifying antioxidant enzymes (Wong *et al.,* 2001; Siraj and Rani, 2003; Wang *et al.,* 2008). Malondialdehyde (MDA) is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Jamil, 2001; Pandey *et al.,* 2001, 2003; Nicholas *et al.,* 2006).

Among the various effects induced by Pb, Zn and Cd in biological systems, the oxidative destruction of membrane polyunsaturated fatty acids, a phenomenon termed lipid peroxidation (LPO), has been observed in numerous tissues both *in vitro* and *in vivo* (Lima *et al.,* 2006), despite the apparent incapacity of Pb, Zn and Cd to directly generate free radicals under physiological conditions (Ebru and Mesut, 2002). Pb, Zn and Cd induced increase in LPO is associated with reductions in CAT activities (Winston and Di Guilio, 1991), and with reduced GSH levels (Younes and Siegers, 1980). Oxygen radical scavengers such as CAT and GSH are protective against lead, zinc and cadmium -induced oxidative damage in fish (Basha and Rani, 2003). Recently, there have been several reports extolling the protective effects of GSH and CAT in preventing peroxidative injury induced by a wide variety of toxic agents (Khessiba *et al.,* 2001). GSH is the most important non protein thiol in living systems (Ebru and Mesut, 2002). Furthermore, reduced GSH is a potent factor in controlling lipid peroxidation (Siraj and Rani, 2003). Pb and Cd are known to have a high affinity for thiol and sulfydryl groups (Younes and Siegers, 1980; Winston and Di Guilio, 1991; Suhel *et al.,* 2006; Kern *et al.,* 2007). Defence systems that tend to inhibit oxyradical formation include antioxidant enzymes such as GSH and CAT, investigated in this research, which are of critical importance in detoxification of radicals to non-reactive molecules, and the low-molecular weight antioxidant GSH. MDA levels are investigated to determine the extent of LPO due to the effect of toxic metal pollutants. Lipid peroxidation or the oxidation of polyunsaturated fatty acids is a very important consequence of oxidative stress for biochemical markers (Viarengo *et al.,* 2000, Choi and Oris, 2000; Yuanyuan *et al.,* 2006; Nicolas *et al.,* 2006).

The present study aiming to investigate the responses of antioxidant, GSH, MDA and CAT levels may provide a useful tool to assess the biochemical effects of battery-manufacturing effluent containing heavy metals on the gills, liver and kidneys of C. *punctata*. To examine the endogenous antioxidant GSH and MDA status, symptoms of cellular toxicity on C*hanna punctata* (Bloch, 1793) exposed to battery-manufacturing effluent containing different concentrations of metals were investigated.

MATERIALS AND METHODS

Chemicals

Ethylene diamine tetra acetic acid (EDTA), sodium azide, glutathione reductase (GR), total reduced GSH, hydrogen peroxide (H_2O_2), 1-chloro, 2,4, dinitrobenzene (CDNB), sulfosalicylic acid, 2,5, dithiobis-tetranitrobenzoic acid (DTNB), nicotinamide adenine dinucleotide phosphate reduced (NADPH) from phenazonium methosulfate and ulfosalicylic acid were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA) and other routine chemicals and reagents (analytical grade) were purchased from local chemical supplier.

Fish and acclimation

Studies were conducted on freshwater fish, *C. punctata*, obtained from local water bodies. The local water bodies were pH 6.9, contained total dissolved solids 145, total hardness 240, calcium hardness 106, dissolved oxygen 3.6, chloride ion 83, alkalinity 110, Na 25 and K 6 mg L^{-1}. Healthy and live specimens of male fish average weight 50 g and average length in the range 15–17 cm. Fish were maintained in 60-L water glass aquaria under standard maintenance procedure during acclimation and exposure (Clesceri *et al.,* 1998). Fifteen fish in one aquarium were acclimatised for 15 days before use in the experiment. Water was kept at oxygen-saturation by aeration and the temperature was also maintained at ambient laboratory temperature (25±2°C). Water in the glass aquaria was changed every 24 h to minimise contamination from metabolic wastes and minimise metal loss just after feeding to reduce contaminant of the environment. Fish were fed autoclaved synthetic goat liver five times a week. Fish were exposed to a battery-manufacturing effluent to find LC_{50}. After exposure, the static acute toxicity of fish was found at 74h.

Experimental setup

The battery manufacturing effluent collected from Sungai Lambing, Malaysia in January 2010, contained heavy metals Pb, Zn and Cd at concentrations 0.25, 0.80 and 0.20 mg L^{-1}, respectively. Physico-chemical characteristics of the effluent were pH (6.3), COD (867), DO (4.3), BOD (93), alkalinity (24), chloride (895) mg L^{-1}. After acclimatisation for 15 days, fish were divided into five groups (n = 5). One group served as control and the other four as exposed groups for 74h durations, and five replicated for each experiment were taken from each group. For convenience, the amendments of battery-manufacturing effluent are denoted as control (0.0% effluent + 100% tap water), 25% (25% effluent + 75% tap

water), 50% (50% effluent + 50% tap water), 75% (75% effluent + 25% tap water) and 90% effluent (90% effluent + 10% tap water). The experiment was planned so that fish from all the groups were sacrificed on the same day.

Metal analysis

The 74h static acute toxicity test was applied to battery manufacturing effluents using minor modifications according to the guideline for wastewater analysis (Clesceri *et al.,* 1998). Five tank replicates of each groups and number of fish (n = 15) were used for each experiment and in the control groups. The soft tissues were removed using Teflon coated spatulas. They were then dried in the digital oven maintained at 75 ^{0}C for 1h. Then the fish tissues of 0.5 g were acidified with HNO_3 and digested in a microwave oven for 32 min and then re-digested with 2 mL of 30% H_2O_2 for 10 min. After digestion, the sample was filtered to remove foreign particles through a Whatman 41 filter paper, diluted to 50 mL with double deionised water and transferred to polypropylene tubes. Pb, Zn, and Cd were analysed using a Perkin-Elmer atomic absorption spectrometer Model 3100. The fish digestion was also performed by adding standard metals. The metal content of these digested samples was determined as above and mean recoveries of Pb, Zn and Cd were 97±5%, 95±4%, 92±4% respectively.

Preparation of postmitochondrial supernatant (PMS)

After the 72 h exposure was complete, fish were anesthetised briefly immersed in MS222 (tricaine methanesulfonate, Sigma-Aldrich, USA) and dissected. The gills, liver and kidneys of the sacrificed fish were quickly removed, cleaned of extraneous tissue, and immediately, immersed in ice-cold saline. The tissues were homogenised in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%), using a Potter–Elvehjem homogeniser. The homogenate was centrifuged at 10,500g for 30 min at 4°C to obtain the PMS, which was used for various biochemical analyses.

Lipid peroxidation (LPO)

Lipid peroxidation was determined by the procedure of (Utley *et al.,* 1967). Levels of LPO were measured by generation of thiobarbituric acid (TBARS)–MDA reactive species, which were referred to MDA equivalents. Tissues were homogenised using 100 mM potassium phosphate buffer (pH 7.2) and centrifuged at 10,000g for 5 min at 4 ^{o}C. The reaction mixture contained: 11.4% of homogenate, 4.6% of 10.6 mM sodium dodecyl sulfate (Sigma-Aldrich) with 0.1 mM butlylated hydroxytoluene (Sigma-Aldrich), 40% of 20% acetic acid (Merck 1.00062) (pH 3.5), 40% of 22.2 mM thiobarbituric acid (Sigma), and 4% of nanopure water in a final volume of 3 mL. The reaction mixture was heated in a 95 ^{o}C water bath for 1h. Once cold, 175 µL of nanopure water and 875 µL n-butanol (Sigma-Aldrich) and pyridine (Sigma-Aldrich) (15:1 v/v) were added through vortexing. Following centrifugation at 10,000g for 5 min, the immiscible organic layer was removed and its absorbance measured at 530 nm. The rate of LPO was expressed as nanomoles of thiobarbituric acid reactive substance (TBARS) formed/h/g of protein using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Non-enzymatic antioxidants

Reduced glutathione was determined using the method of Sedlak and Lindsay (1968) and studied as nonenzymatic antioxidants. GSH was determined in PMS which was precipitated with sulfosalicylic acid (4.0%) in a ratio of 1:1. The samples were kept at 4°C for 1 h and then subjected to centrifugation at 1200g for 15 min at 4°C. The assay mixture contained supernatant, 0.1 M phosphate buffer, and 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) (stock=100 mM in 0.1 M phosphate buffer) in a total volume of 3 mL. The optical density of reaction product was read at 412 nm on a spectrophotometer and results were expressed as nmol GSH g^{-1} tissue.

Antioxidant enzymes

CAT activity was assayed by the method with some modification as described by Ahmad *et al.,* 2000). The assay mixture consisted of 0.1M phosphate buffer (pH 7.4), 0.019M hydrogen peroxide and 10% PMS in a final volume of 3 mL. CAT activity was calculated as nmol H_2O_2 consumed/(min mg^{-1} protein). The reaction mixture consisted of 0.1M phosphate buffer, 1 mM GSH, 1 mM CDNB and PMS (10%) in a total volume of 2 mL. The enzyme activity was calculated as nmol CDNB conjugates/(min mg^{-1} protein) using amolar extinction coefficient of $9.6\times103M^{-1}$ cm^{-1}. Glutathione peroxidase (GPx) activity was assayed according to the method described by Ahmad *et al.* (2000) with some modifications. The assay mixture consisted of 0.1M phosphate buffer, 1mM EDTA, 1mM sodium azide, GR (1 IU mL^{-1}), 1 mM GSH, 0.2 mM NADPH, 0.25 mM H_2O_2 and PMS (10%) in a total volume of 2 mL. The enzyme activity was calculated as nmol NADPH oxidised/(min mg^{-1} of protein), using a molar extinction coefficient of $6.22\times103M^{-1}$ cm^{-1}. Catalase activity was calculated in terms of nmol H_2O_2 consumed/min mg^{-1} protein.

Statistical analysis

The results were expressed as mean values ± SD. The significances between mean values were determined according to the Mann-Whitney U test evaluated by the SPSS (version 6.0) computer program. The P <0.05 level was chosen as statistically significance throughout. Statistical differences cannot be just between each dose independently and the controls. It would make a significant improvement to the analysis to make statistical comparisons between the different exposure treatment and tissues.

RESULTS

The 90% amended effluents showed the highest significant accumulation of metals in fish tissues as shown in Figures 1–3. The kinetics of metal uptake in various tissues of liver and kidney of C. *pucntata* were significantly higher (P<0.05) than in the gills (P<0.01) (Figures 1–3). In fish, metal accumulation in the gills was lower than in the liver and kidney. Pb (6.87 µg g^{-1}) was significantly more in the liver, while Cd (6.97 µg g^{-1}) was accumulated in the kidney. The accumulation of metals was higher in the liver compared to that in the gills and kidney (liver> kidney> gills), while Zn (4.86 µg g^{-1}) had the highest accumulation in gills compared with the liver and kidney that showed the lowest accumulation (P<0.01).

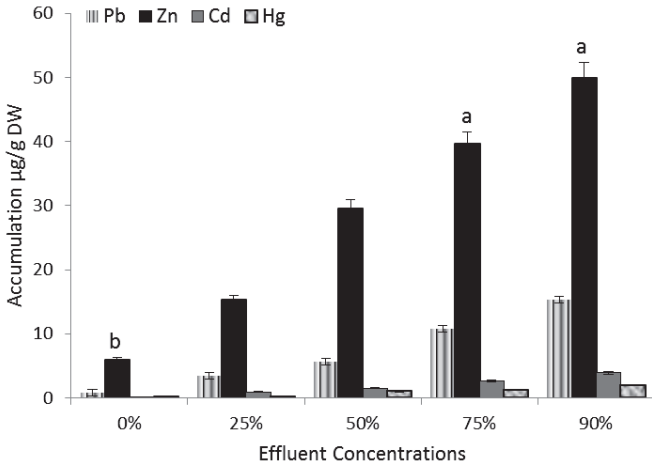

Figure 1 Accumulations of metals (µg g^{-1} DW) in gills show how they are affected by amendments of battery manufacturing effluent. a Indicates significant and b non-significant values. All values are means of five independent experiments (five replicates each), the vertical bars indicate the standard deviations. P <0.05 strongly significant, P <0.01 less significant with respective to control.

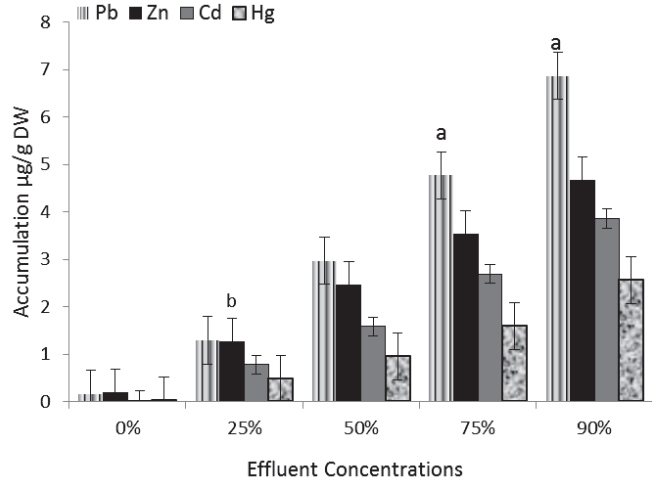

Figure 2 Accumulations of metals (µg g^{-1} DW) in liver show how it is affected by amendments of battery manufacturing effluent a Indicates significant and b nonsignificant values. All values are means of five independent experiments (five replicates each), the vertical bars indicate the standard deviations. P <0.05 strongly significant, P <0.01 less significant with respective to control.

Biochemical analysis

In this study, most antioxidant enzymes showed a decrease their activities in fish exposed to battery-manufacturing effluent over a period of 74h (Figures 4–6). Lipid peroxidation in exposed fish increased by 62% and 42.6% and 19.8% in the kidneys, liver and gills (P<0.05, P<0.05 and P<0.01) compared to control (Figure 1). Reduced GSH contents were significantly decreased in the liver, kidneys and gills of exposed fish, (P<0.05, P<0.05 and P<0.01) (Figure 2). There were no significant changes in the levels of CAT activity in the gills, liver and kidneys (Figure 3) when compared to control. CAT values decreased (P<0.01) in the gills, liver and kidney by the end of the experiment as a consequence of the amendments (Figure 3). CAT activity in the gills, liver and kidney of fish exposed to different amounts of effluent affected 7.8% gills, 21.9% liver and 31% kidney compared to control (Figure 3).

DISCUSSION

Metal accumulation from industrial effluent in fish tissues occurs in relation to aquatic environmental concentrations, duration of exposure and characteristics of the water (Ahmad *et al.,* 2000). The accumulation of the metals Pb and Hg was found to depend on the exposure to effluent concentrations. The metal availability in kidneys and liver was more than in the gills at 90% effluent (Canli 2001). Pb and Cd-induced peroxidative injury in these organs is mediated via lipid peroxidation, reflected by significant reductions in reduced GSH and CAT activities (Figures 5 and 6). Exposure of fish to the effluent which contained metals showed that catalase did not increase, whereas GSH and MDA increase many-folds in the 75% and 90% amendments. In fish exposed to battery effluent for 74h, the levels of MDA in the liver and kidney,

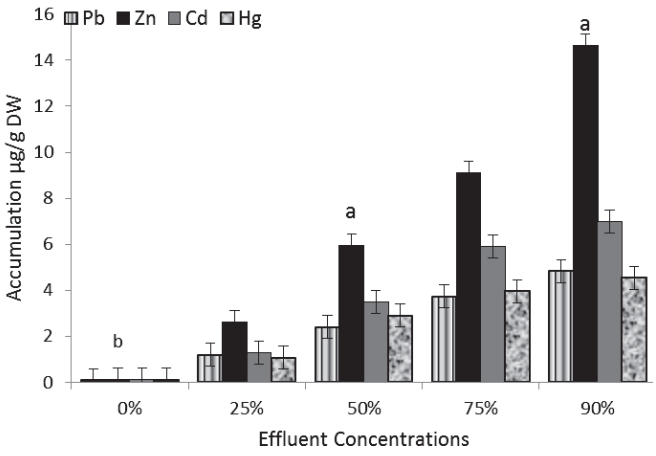

Figure 3 Accumulations of metals (µg g^{-1} DW) in kidneys show how they are affected by amendments of battery manufacturing effluent. a Indicates significant and b nonsignificant values. All values are means of five independent experiments (five replicates each), the vertical bars indicate the standard deviations. P <0.05 strongly significant, P <0.01 less significant with respect to control.

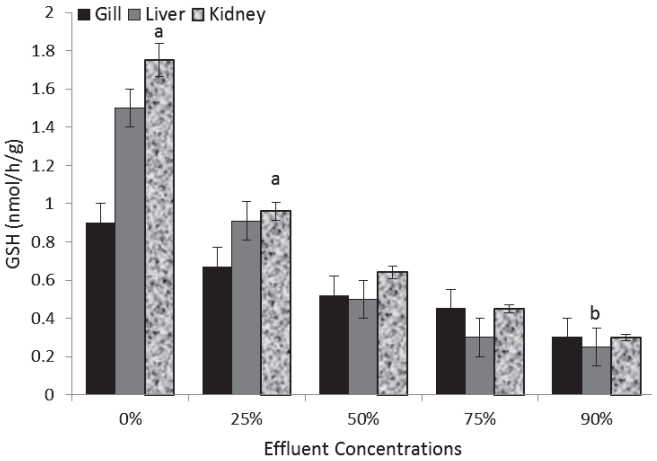

Figure 4 GSH activities in gills, liver and kidneys showing how they are affected by amendments of battery-manufacturing effluent. a Indicates significant and b non-significant values. All values are means of five independent experiments (five replicates each), the vertical bars indicate the standard deviations. P <0.05 strongly significant, P <0.01 less significant with respect to control.

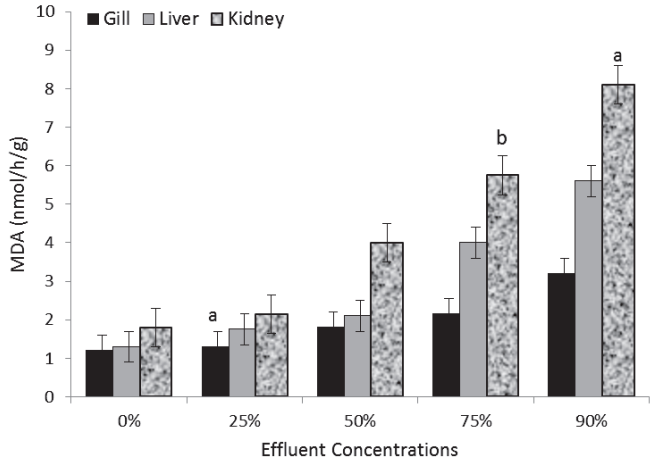

Figure 5 MDA activities in gills, liver and kidneys showing how they are affected by amendments of battery-manufacturing effluent. a Indicates significant and b non-significant values. All values are means of five independent experiments (five replicates each), the vertical bars indicate the standard deviations. P <0.05 strongly significant, P <0.01 less significant with respect to control.

estimated according to the detected amount of MDA, was about 40% (75% effluent) and 70% (90% effluent) higher than compared to control (0.0% amendment) (Figure 4). Thus, it appears that a period of 74h is sufficient for the development of oxidative damage in tissues. Our findings are similar to other research (Pandey *et al.,* 2001; Prakash and Jagannath Rao, 1995; Kelly *et al.,* 1998; Siraj and Rani, 2003, Viarego *et al.,* 2000; Santos *et al.,* 2004). Pb and Cd-toxicity, induced increases in superoxide anion radical production and lipid peroxidation and nonenzymatic scavengers have also been reported (Suhel *et al.,* 2006; Winston and Di Guilio, 1991; Younes and Siegers, 1980). The data presented in this paper showed that Pb, Zn and Cd, induce peroxidative injury in the liver and kidneys but not in the gills (Fasulo *et al.,* 2008).

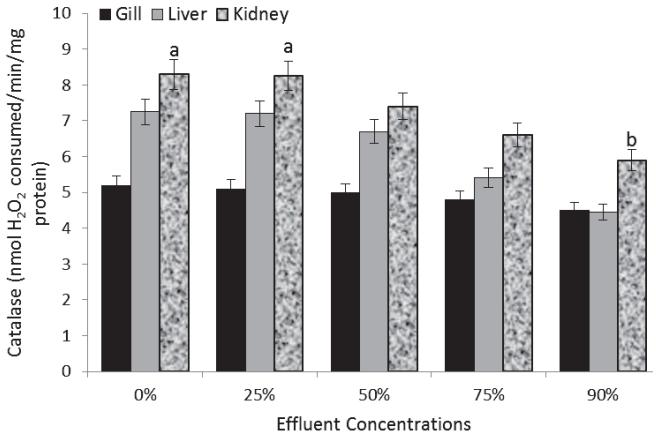

Figure 6 Catalase activities in gills, liver and kidneys showing how they are affected by amendments of battery-manufacturing effluent. a Indicates significant and b nonsignificant values. All values are means of five independent experiments (five replicates each), the vertical bars indicate the standard deviations. P <0.05 strongly significant, P <0.01 less significant with respect to control.

In this study, increased free radical generation was found in C. *punctata* under amendments of effluent containing heavy metal stress as indicated for MDA production, which is similar to the effect of heavy metals (Stohs *et al.,* 2000). This suggests that the toxic effect of heavy metals is probably exerted through free radical generation. In higher animals, heavy metals induce generation of superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-), and singlet oxygen ($^1O_2$)**,** collectively termed ROS, and exert a variety of damaging effects, also called oxidative stress (Ebru and Mesut, 2002; Ferreira *et al.,* 2010). The ROS can rapidly attack all types of biomolecules such as nucleic acids, proteins, lipids, and amino acids (Lima *et al.,* 2006), leading to irreparable metabolic dysfunction and cell death. Many redox-active and non-redox-reactive metals are known to cause oxidative stress, as indicated by lipid peroxidation and H_2O_2 accumulation in the cells (Pandey *et al.,* 2001). ROS can act on membrane lipids leading to lipid peroxidation and to the formation of MDA. However, GSH and catalase could protect cells against toxic effects of ROS (Basha and Rani, 2003; Ebru and Mesut, 2002). Furthermore, lead, zinc and cadmium are redox-inactive metals which inactivate the cellular antioxidant pool and disrupt the metabolic balance, thus enhancing the load of ROS (Stohs *et al.,* 2000). The acute toxicity of lead and cadmium to the fish may be due to the kinetics of metal uptake in various tissues in the liver and kidneys, and the relatively mild toxicity of zinc may be due to its slow uptake or less toxicity.

The level of MDA in the tissue is considered a measure of lipid peroxidation status. Lipid peroxidation is linked to the production of O^{-2}. Thus, the increased level of MDA suggests that metal ions stimulate free radical generating capacity of the fresh water fish. When the levels of ROS formed exceed, the ability of the antioxidant system to cope with damage to cellular components occurs. Lead and cadmium has a strong affinity for sulfydryl groups and can deplete GSH and protein-bound sulfhydryl groups, resulting in MDA contents

increase and enhanced production of reactive oxygen species such as superoxide, hydroxyl radical and hydrogen peroxide (Livingston, 2001; Wang *et al.,* 2008). It has been reported that the hepatic toxicity of Pb and Cd may be due to its binding to intracellular sulfydryl groups and that intracellular GSH levels may provide protection against metal cytotoxicity in the liver, kidneys and gills (Winston and Di Guilio, 1991; Franco-Ura *et al.,* 2010). According to the experimental results, the GSH levels in the liver and kidneys of effluent amendment fish were significantly lower than in controls (Figure 5) due to the GSH actively participation in free radicals detoxification. Figure 6 shows that the CAT activity decreased in the gills, liver and kidney of the experimental group compared with the control group. CAT activity was about 12% and 10% lower in the liver and kidneys of fish exposed to lead, zinc and cadmium compared with the control. Similarly, GSH is more strongly reduced than CAT activity in effluent amended C. *puntata*. The reduction in CAT activity, along with a decrease in reduced GSH levels in fish exposed with increasing effluent amendments is probably due to a decreased accumulation of zinc, required for the activity of these enzymes. Similar findings were made by other researcher (Jamil, 2001; Suhel *et al.,* 2006; Winston and Di Guilio, 1991; Younes and Siegers, 1980).

The results from the experiments show that the production of MDA was observed in the gills, liver and kidneys exposed to the effluent during 74h. These results are similar to those obtained in Pb, Zn and Cd exposed fish, C. *punctata*. Pb, Zn and Cd have high affinities for reduced GSH which is the primary intracellular antioxidant agent and can cause the irreversible excretion of GSH leading to the depletion of GSH and the increase of MDA. However, in the gills, liver and kidney of C. *punctata* exposed to effluents, a decrease in MDA levels and an increase in CAT levels could produce antagonistic effects where the decrease could result in an intensification of antioxidant systems including CAT, limiting the MDA formation. A relationship between GSH consuming the oxide radicals and MDA enhancement was shown in the C. *punctata*, in response to effluent amendments. This increase of MDA and decrease of GSH, CAT could result in a saturation of antioxidant systems on 90% effluent amendment.

CONCLUSION

MDA and GSH–CAT maybe as useful tools for risk assessment due to the toxicity of metals from industrial effluent. The accumulation of metal Pb in liver was found to be more significant and MDA levels create excess LPO in 90% effluent. Whereas the lowest accumulation of Pb was found in the kidneys and the highest accumulation of Cd was more significant in the 90% effluent. These data suggest that GSH may be more important than CAT in preventing peroxidative injury due to the toxicity of Pb, Hg and Zn in the gills, liver and kidneys. The results indicate that the accumulation of metals from 75 to 90% effluent uses oxidative stress and antioxidant biomarkers like MDA and GSH–CAT, whereas 25 to 50% effluent shows non-significant MDA and GSH–CAT activity. The toxicity evaluation of battery manufacturing effluent suggest that this technique may be used as an alternative approach for biomarkers of heavy metals in industrial effluents and wastewaters

ACKNOWLEDGEMENTS

The authors thank the deanship of scientific research (DSR) and King Saud University (KSU) for its funding of this research through the research group project no. RGV-VPP-316.

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