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Research Article

Effects of Water-Borne Mercury and Cadmium Exposure on Lipid Peroxidation and Antioxidant Enzymes in Mangrove Red Snapper *Lutjanus argentimaculatus*

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Abstract: Effects of waterborne cadmium (Cd^{2+}) and mercury (Hg^{2+}) both separately and in combination on the lipid peroxidation and antioxidant activity in *Lutjanus argentimaculatus* was investigated. The fish was exposed for 3, 7 and 15 days respectively to Cd^{2+}, Hg^{2+} and the mixture of both. Exposure to Cd^{2+} was done at three different concentrations *viz* 1, 5 and 100 μg/L .The fish was exposed to Hg^{2+} at 0.2, 0.5 and 10 μg/L. Further *L. argentimaculatus* was also exposed to a mixture containing 5 μg/L Cd^{2+} and 0.5 μg/L Hg^{2+}. The results showed increased levels of antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POD) ($p<0.05$) both in hepatic and branchial tissues. The level of Malonialdehyde (MDA) which is an indicator of lipid peroxidation also showed significant increase ($p<0.05$). Further, antioxidant enzymes and MDA could not fall down to normal levels even after 15 days of release to clean sea water in all the treatments tested. However, the activity of antioxidant enzymes in the fishes exposed to mixture containing both Cd^{2+} and Hg^{2+} did not showed remarkable raise as when treated separately. The study indicated that the increase of antioxidant enzymes activity and MDA need to be considered carefully as pollution indicators as their values do not conform well to the corresponding metal ion concentrations, in view of co-effects of metals.

Keywords: Antioxidant enzymes, biomarkers, *Lutjanus argentimaculatus*, trace metal

INTRODUCTION

In recent decades, rapid growth of the economy in China has been coupled with increasing environmental pollution and high metal concentrations are observed in the sediments, water and organisms collected in estuarine and coastal waters (Zhou *et al*., 2008; Pan and Wang, 2012). The elevated levels of metal contamination along China's coastal environment can increase the risk of metal exposure to humans through seafood consumption (Pan and Wang, 2012), of which the cadmium and mercury have raised the alarm for the public and the authorities, so the Program Integrated Prevention and Control Planning of Heavy Metal Pollution has become the top most priority to be fulfilled in China.

Heavy metals such as cadmium and mercury, have no known biological significance(Rousse *et al*., 1998; Bebianno *et al*., 2005), except for their high acute and potential toxicity (Wolf and Baynes, 2007), which can be bioaccumulated in the aquatic organisms, magnified in the food chain, thus threatening human health and the integrity of aquatic ecosystems (Zhou *et al*., 2008).

Fishes are the top position in the aquatic food chain and an important food source (Agah *et al*., 2009) and the consumption of marine fish is one of the primary pathways of exposure to Hg and Cd for humans (Pan and Wang, 2012). Understanding the current effect of pollution to economic importantly aquatic organisms is crucial to China's seafood industry, as both the domestic demands and export of seafood have increased dramatically recently (Gao and Gao, 2005; Lindkvist *et al*., 2008).

Mangrove Red Snapper *Lutjanus argentimaculatus,* is one of the most importantly economic fishes in South China Sea coastal waters, especially in marine cage culturing industry, while metals contamination as mercury or cadmium may be directly linked to fish biological process (growth, mortality, maturity, etc.) and the healthy seafood production.

It is known that metals result in oxidative stress by producing Reactive Oxygen Species (ROS), e.g., superoxide anion radical ($O_2^{\cdot -}$) and H_2O_2 (Wu *et al*., 2010). Over-accumulation of ROS leads to cellular peroxidation and molecular damage (Kehrer, 1993).

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Table 1: Experimental design

Group	CK	I	II	III	IV	V	VI	VII
Kinds of metal	Nil	Cd^{2+}	Cd^{2+}	Cd^{2+}	Hg^{2+}	Hg^{2+}	Hg^{2+}	Cd^{2+}+Hg^{2+}
Concentration (μg/L)	0	1	5	100	0.2	0.5	10	5+0.5

However, for ions of cadmium and mercury, mechanisms leading to alterations in cellular redox homeostasis are poorly understood (Banerjee *et al*., 2001; Abdollahi *et al*., 2004). The antioxidant machinery is composed of enzymes and nonenzymatic components and the enzymes include ROS scavengers like Superoxide Dismutase (SOD), Peroxidase (POD) and Catalase (CAT) (Khan and Kour, 2007). Such enzymatic-based anti-oxidative system is one of the important strategies for fish to respond to environmental stresses. In most cases, toxins can give rise to the increase activities of antioxidant enzymes which reflects not only the degree of toxicity but as well the ability to tolerate the stress (Wu and von Tiedemann, 2002; Peixoto *et al*., 2006; Chen *et al*., 2011). However, little is known about the adverse effect of metals on prospective cage culturing fish *Lutjanus argentimaculatus*. The aim of this study is to evaluate the effects of cadmium and mercury on the antioxidant responses in *Lutjanus argentimaculatus* and to achieve a better understanding of the biological mechanisms for mercury and cadmium induced oxidative stresses in marine fishes and assess the application of these biomarkers *in situ* for the better water quality management in Mari culture systems.

MATERIALS AND METHODS

Analytical grade Mercury chloride ($HgCl_2$) and Cadmium chloride ($CdCl_2$) were purchased from Tianjin Chemical Corp, China, with purity greater than 99%. All other chemicals were of analytical grade and obtained from commercial sources. Young red snappers *Lutjanus argentimaculatus* (average body weight 3.87±0.69 g) were procured from hatchery base of Shenzhen city, Guangdong, China and acclimatized to the laboratory in aquarium tanks containing clean seawater for 7 days. The tanks were aerated to provide healthy environment. During acclimatization and experimentation, the animals were fed once in 48 h with the formulated pellet feed containing 40% protein at 5% of the body weight. The water in the experimental tanks was renewed half after every 48 h.

After acclimatization, fishes of uniform size were randomly selected and divided into eight groups for seven treatments and the control as per the experimental design shown in Table 1. The temperature was recorded using a thermometer and the pH was recorded using a pH meter. The salinity was recorded using YSI 550A Analyzer.

Experimental fishes were selected from acclimatized groups at the rate of 60 fishes per treatment. They were reared in circular tanks holding 500 L of sea water. About 10 fishes were sampled on 3rd, 7^{th} and 15^{th} day of exposure respectively to estimate the levels of superoxide dismutase (SOD), Catalase (CAT) and Peroxidase (POD) and Malonialdehyde (MDA) both in hepatic and bronchial tissues. The surplus fish under each treatment were transferred to clean sea water tanks and sampled on 30th day (i.e. 15 days after the day of exposure).

For biochemical analysis, the hepatic and branchial tissues of the sampled fish in each treatment were dissected rapidly and washed in ice-cold normal saline (0.9%, w/v), blotted, flash frozen in liquid nitrogen and stored at -80˚C until analysis. All the procedures involved in the preparations of microsomes and purification of enzyme were performed at 0-4˚C. Fresh tissues (0.4 g) frozen in liquid nitrogen were ground and dissolved in 4 mL of the ice-cold 10 mM Tris-HCl homogenizing buffer (pH 7.4). The homogenate was centrifuged at 4,000 g for 30 min at 4˚C. The supernatant was immediately analyzed for SOD (Oyanagui, 1984), CAT (Aebi, 1974), POD (Upadhyaya *et al*., 1985), MDA (Ohkawa *et al*., 1979) and protein content (Braford, 1976) using a Bovine Serum Albumin (BSA) as a standard. Statistical analyses were performed using the statistical package SPSS 13.0. Significant differences between the treatments were statistically evaluated using one-way ANOVA.

RESULTS AND DISCUSSION

During the experimental period, there was no significant difference in the mortality of fishes between the treatments. The water temperature was 23±1˚C.pH was 7.7±0.1 and Salinity was 34±1. The results of SOD, CAT, POD and MDA after exposures to Cd, Hg and Cd and Hg mixture and 15 days withdrawal are shown in Fig.1, 2, 3 and 4 respectively.

Figure 1 shows the effects of Cd, Hg and their mixture on SOD activity in the hepatic and brachial tissues of *L. argentimaculatus*. After 3 days of exposure, both metals induced the level of SOD in relation to the control animals, although no significant differences could be observed due to various concentrations of Cd, Hg and mixture ($p>0.05$). The maximum activity recorded in the hepatic and branchial tissues were 38.76 U/mg proteins, 13.94 U/mg proteins, respectively. With the increase of exposure time (7 days and 15 days), the SOD activity was significantly induced at higher concentration of metals both in the hepatic and branchial tissues ($p <0.05$). After 15 days of

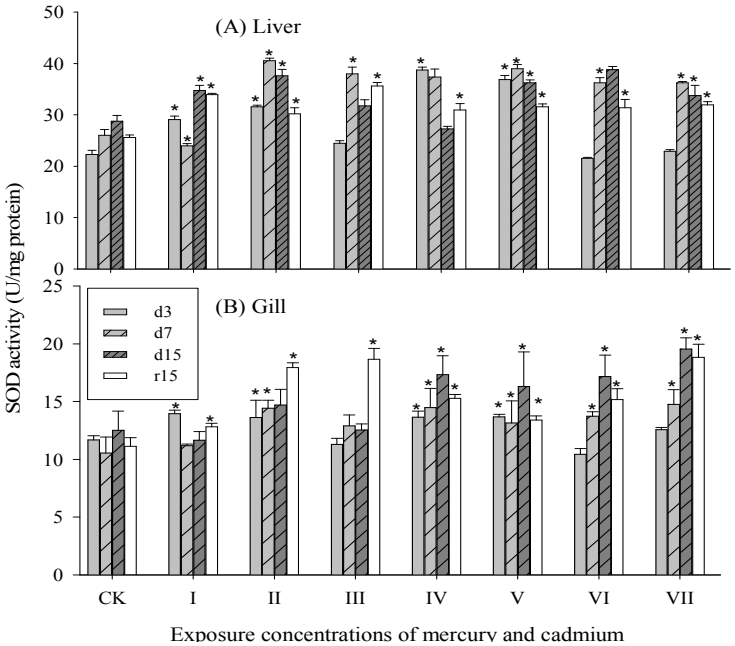

Fig. 1: Activities of SOD enzymes in hepatic and branchial tissues form *Lutjanus argentimaculatus* under treatments of cadmium and mercury, of which d3, d7, d15, r15 were exposure of 3,7,15 days and releases of 15 days respectively. Values were expressed as mean ±SD. *p<0.05

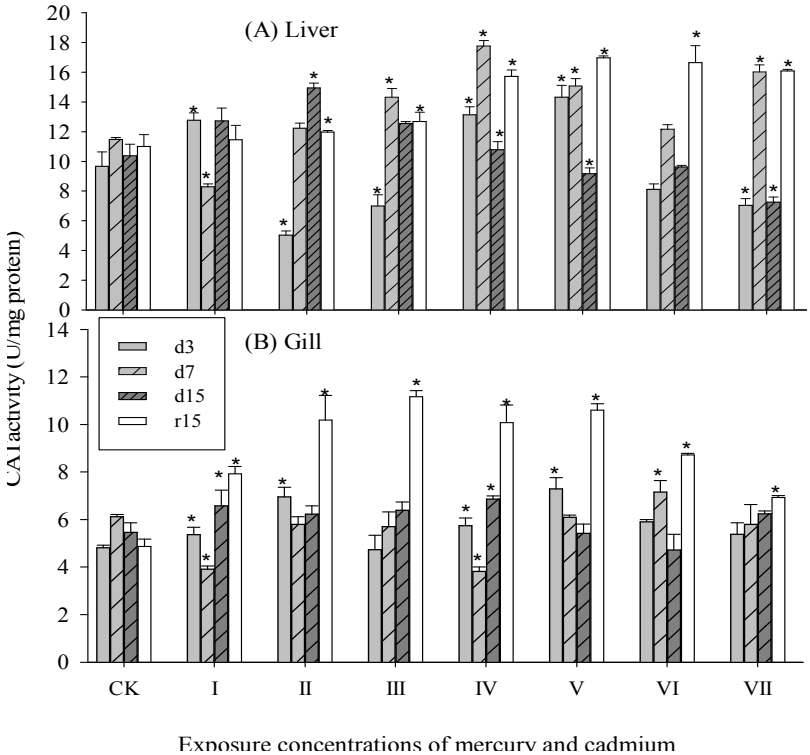

Fig. 2: Activities of CAT enzymes in hepatic and branchial tissues from *Lutjanus argentimaculatus* under treatments of cadmium and mercury, of which d3,d7,d15,r15 were exposure of 3,7,15 days and releases of 15 days respectively. Values were expressed as mean ±SD. *p<0.05

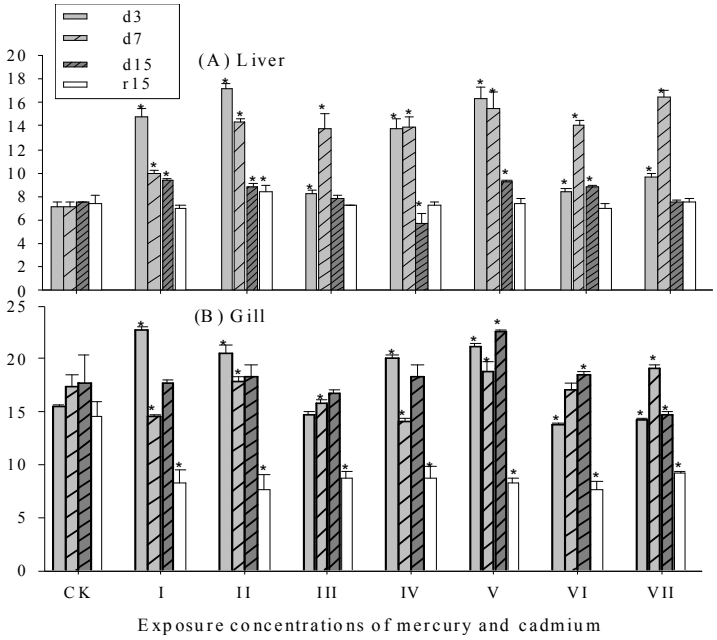

Fig. 3: Activities of POD enzymes in hepatic and branchial tissues from *Lutjanus argentimaculatus* under treatments of cadmium and mercury, of which d3,d7,d15,r15 were exposure of 3,7,15 days and releases of 15 days respectively. Values were expressed as mean ±SD. *p<0.05

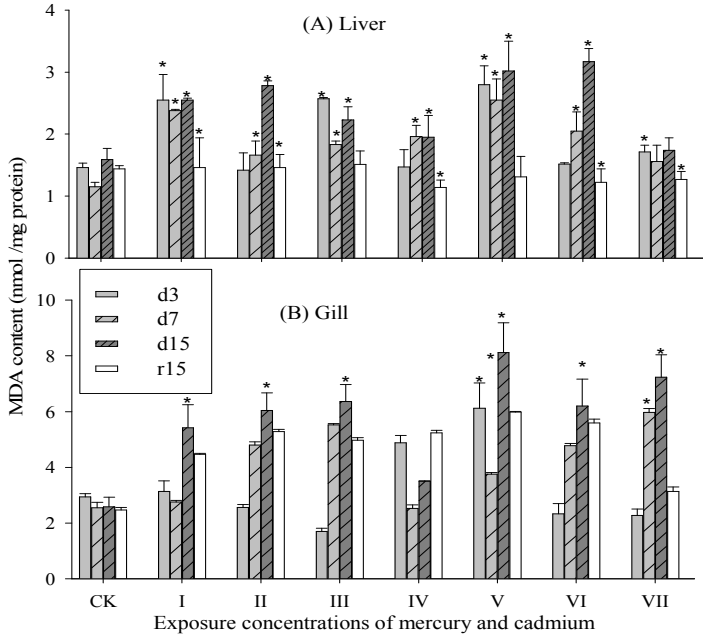

Fig. 4: Levels of LPO in hepatic and branchial tissues from *Lutjanus argentimaculatus* under treatments of cadmium and mercury, of which d3,d7,d15,r15 were exposure of 3,7,15 days and releases of 15 days respectively. Values were expressed as mean ±SD. *p<0.05

withdrawal period, the activities of SOD both in liver and gill with respect to Cd (100 ug/L) were still at a higher level (31.94 U/mg proteins and 18.65 U/mg proteins respectively). As far as mercury was concerned, the activities of SOD in liver and gill with respect to Hg (5 ug/L) were also still at a higher level (31.39 U/mg proteins and 15.17 U/mg proteins respectively). In the case of treatment VII (Cd and Hg

mixture), the activities of SOD in liver and gill with respect to Cd (5 ugCd/l+0.5 ugHg) were also still at a higher level (31.94 U/mg proteins and 18.81 U/mg proteins respectively). It is evidenced from the above results that after 15 days of exposures on Cd, Hg or mixture, the 15 days release is not yet enough to return to normal SOD level.

The results relative to CAT in gill and liver tissues were shown in Fig. 2. The CAT activity in the liver was induced after 3 days of exposure to 1 μg/L Cd^{2+} and concentrations of Hg^{2+} and inhibited on concentrations of 5μg/L Cd^{2+} and 100 μg/L Cd^{2+}. Then the activity of CAT was induced significantly, even after 15 days release after exposure, which showed the similar trends to the variation of SOD in corresponding tissues and exposure time. The activity of CAT in the gill under treatment of mixture (5μg/L Cd^{2+}+0.5μg/L Hg^{2+}) did not vary as the treatment II and V, indicating the mixture of $Cd^{2+}$and Hg^{2+} decreased their toxicity by the antagonism of ions.

The response of POD in both hepatic and branchial tissues was shown in Fig. 3. After 3 days of exposure, statistically significant induction of POD activity in both tissues was recorded. With the increase of exposure time to 7 days, the activity of POD was inhibited in treatments of I, IV, VII, but still induced in higher treatments of II, III, V and VI in branchial tissue. As for the hepatic tissue, the activity of POD was induced significantly. On 15 days of exposure, the POD activity of branchial in treatments had no significant variation when compared to the control, with the exception of slight decrease in group VII. The POD activity in hepatic tissues increased in group I, II, IV and V had no significant variation. Unlike the variation of SOD and CAT, the activities of POD in the gill were inhibited after 15 days of release and had no significant difference in the liver. It indicated that the POD activities can recover shortly after the release of exposures of Cd, Hg and mixture and the activity of POD in liver was not sensitive as SOD and CAT.

Figure 4 reported the levels of MDA in both tissues exposed to Cd, Hg and mixture. In general, a higher and significant accumulation of MDA was registered in both tissues, even on the 15 days of released exposure, which showed similar induction to the SOD and CAT. In general terms of overall tissue specificity, the rate of induction/inhibition of SOD and CAT were higher in gill tissues than in hepatic tissues and both of their activities were induced more than the activities of POD. The levels of MDA in the hepatic tissues under treatments of mixture were lower than in its corresponding single metals exposure (group II and group V) and returned to normal level faster in gills under mixture administration.

The general similarities between changes in activities of SOD, CAT, POD and MDA content at higher metal concentrations suggested that antioxidant response of snapper more sensitive in lower concentrations of heavy metal pollution in short time (such as 3 days of exposure). And the antioxidant system maybe disturbed during 7 days of exposure, additionally with the increase of exposure time (15 days), the oxidative stress from metal exposure became stronger. Antioxidant response plays an antioxidant role in metal-induced lip peroxidation both in the hepatic and branchial tissues.

Results showed that the depletion induced by mercury occurred at lower concentrations was higher when compared to that by cadmium, which could account for the higher activities of SOD in treatments of Hg than in treatments of Cd. With the increase of exposure time, both metals exerted deleterious effect on the antioxidant system and resulted in lip peroxidation both in tissues of gill and liver. After exposure of 15 days, the investigation of relative assays after 15 days release indicated that the antioxidant system still had not recovered to the normal levels.

As for treatment of mixture of Cd and Hg, the effects of mixture on the levels of SOD, CAT and POD, MDA was lower when compared to the corresponding single metal treatment respectively. These effects may reflect some intracellular antioxidant actions, but the major effect is probably attributable to direct metal reactions with the thiol compounds, possibly enhanced by sequestration or antagonism of the metals (Wolf and Baynes, 2007). Similar conclusion that combined Hg and Cd toxicity showed antagonism also was made in conventional acute toxicity assay (Sui *et al*., 1999).

Unlike organic pollutants, Heavy Metals (HM) neither undergo degradation in the aquatic organisms or in the environments. Understanding the toxic effects of HM in fish is both beneficial to the assessment of human health risks and sustainable development of aquaculture. The present results showed that the activities of SOD, CAT, POD and the content of MDA in tissues of liver and gill were affected during 15 days of exposure. Also their variations indicated the difference and possible mechanism of oxidative stress from Cd and Hg between gill and liver tissues.

The divalent metal ions such as mercury, cadmium to cause toxicity to cells and organs has been known for some time (Valee and Ulmer, 1972; Wolf and Baynes, 2007) and a large number of biochemical processes are affected: enzymes are inhibited, nucleic acid conformation is changed and oxidative phosphorylation is affected. Wolf found that both cadmium and mercury ions can induce an oxidative stress through depletion of GSH and inactivation of thiol enzymes and that the

increase in oxidative damage leads to severe endothelial cells dysfunction (Wolf and Baynes, 2007). We conclude that the activities of antioxidant enzymes are the better response of fish antioxidant system to the pollution stresses from Hg^{2+} and Cd^{2+} and at low doses of the metal ions, there is a significant compensatory antioxidant response, characterized by an induction in activities of SOD, CAT and POD.

We investigated that neither the activities of enzymes nor the contents of MDA confirm well with the concentrations of waterborne metal ions. At low metal concentrations, metal response elements and other sensors, signal transcription factors up-regulate the expression of SOD, CAT and glutathione biosynthesis and other antioxidant enzymes. Together, these actions enhance antioxidant defenses (Wolf and Baynes, 2007). Strong protective response at low metal ion concentration would explain threshold responses in metal ion toxicity, i.e., low doses of metals may enhance protective mechanisms, while high doses lead gradually to cytotoxicity (Kroes *et al*., 2004) and inhibit the antioxidant enzymes.

Studies have been done on metal accumulation in different fish species, other aquatic organisms and sediments, which have clearly demonstrated the aquatic environment is facing metal pollution (Bozcaarmutlu and Arnic, 2007), while the conventional methods for metal monitoring by analyzing the concentration of metals in aquatic organisms is far insufficient for the water quality assessment and aquatic toxicology (Issam *et al*., 2003; Waykar and Shinde, 2011). The influence of HM the biochemical effect of fish and biomarkers relative have been studied (Golovanova, 2008), the results of this study showed that the mixture of HM may affect the sensitivity and application of antioxidant enzymes as biomarkers and the response of activity of enzymes even in laboratory only can indicate the trends of variation, which should be carefully considered *in situ* as their values do not conform well with the corresponding metal ion concentrations.

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