J. Ocean Univ. China (Oceanic and Coastal Sea Research)
DOI 10.1007/s11802-015-2464-9
ISSN 1672-5182, 2015 14 (3): 511-521
http://www.ouc.edu.cn/xbywb/
E-mail:xbywb@ouc.edu.cn

Effects of Waterborne Cu and Cd on Anti-oxidative Response, Lipid Peroxidation and Heavy Metals Accumulation in Abalone *Haliotis discus hannai* Ino

LEI Yanju, ZHANG Wenbing*, XU Wei, ZHANG Yanjiao, ZHOU Huihui, and MAI Kangsen

The Key Laboratory of Aquaculture Nutrition and Feed of Ministry of Agriculture; *The Key Laboratory of Mariculture of Ministry of Education*; *Fisheries College*, *Ocean University of China*, *Qingdao* 266003, *P. R. China*

(Received August 23, 2013; revised October 31, 2013; accepted February 8, 2015)

Abstract The aim of this study was to compare the effects of waterborne copper (Cu) and cadmium (Cd) on survival, anti-oxidative response, lipid peroxidation and metal accumulation in abalone *Haliotis discus hannai*. Experimental animals (initial weight: 7.49 g ± 0.01 g) were exposed to graded concentrations of waterborne Cu (0.02, 0.04, 0.06, 0.08 mg L^{-1}) or Cd (0.025, 0.05, 0.25, 0.5 mg L^{-1}) for 28 days, respectively. Activities of the anti-oxidative enzymes (catalase, CAT; superoxide dismutase, SOD; glutathione peroxidases, GPx; glutathione S-transferase, GST), contents of the reduced glutathione (GSH) and malondiadehyde (MDA) in the hepatopancreas, and metal accumulation in hepatopancreas and muscles were analyzed after 0, 1, 3, 6, 10, 15, 21, 28 days of metal exposure, respectively. Results showed that 0.04 mg L^{-1}, 0.06 mg L^{-1} and 0.08 mg L^{-1} Cu caused 100% death of abalone on the 21st, 10th and 6th day, respectively. However, no dead abalone was found during the 28-day waterborne Cd exposure at all experimental concentrations. Generally, activities of SOD and GST in hepatopancreas under all Cu concentrations followed a decrease trend as the exposure time prolonged. However, these activities were firstly increased and then decreased to the control level and increased again during Cd exposure. Activities of CAT in all Cu exposure treatments were higher than those in the control. These activities were firstly increased and then decreased to the control level and increased again during Cd exposure. Contents of MDA in hepatopancreas in all Cu treatments significantly increased first and then decreased to the control level. However, the MDA contents in hepatopancreas were not significantly changed during the 28-day Cd exposure. The metals accumulation in both hepatopancreas and muscles of abalone significantly increased with the increase of waterborne metals concentration and exposure time. These results indicated that *H. discus hannai* has a positive anti-oxidative defense against Cu or Cd. In conclusion, anti-oxidative mechanism in abalone to resist waterborne Cu did not follow the same pattern as that for waterborne Cd.

Key words abalone; copper; cadmium; anti-oxidation; peroxidation; toxicity

1 Introduction

Heavy metals are the most common pollutants in many coastal areas worldwide. They are of considerable environmental concern for human being due to their toxicity, wide sources, non-biodegradable properties and accumulative behaviors. In China, with the rapid development of the industry, the fossil fuel burning, waste incineration, industrial waste discharge and mining have contributed to heavy metal contamination. It constitutes a continuing threat to aquatic ecosystems. Cadmium (Cd) and copper (Cu), two common types of heavy metal pollutants, exceeded the environmentally allowable content in several bays in China, such as Jinzhou Bay, Bohai Bay and Jiao- zhou Bay (Xu *et al*., 2000; Xu *et al*., 2005; Chen *et al*., 2004).

Cadmium is a non-essential and highly toxic metal to animals. It can be accumulated in marine organisms, and cause a wide range of toxic effects on the cellular, organismal, and population levels (Sörensen, 1991; Goering *et al*., 1995; de La Torre *et al*., 2000). Cu is an essential element, acting at low concentration as a cofactor for important enzymes (Franco *et al*., 2009). However, at high concentrations, it becomes toxic to cause loss of appetite, reduced growth, ion loss, decreased aerobic scope, histological alterations and mortality in aquatic animals (Marr *et al*., 1996; McGeer *et al*., 2000; Handy, 2003; Mazon *et al*., 2004). The toxic effect of heavy metals appears to be related to the production of reactive oxygen species (ROS) (Winterbourn, 1982), and the reduction of the cellular antioxidative capacity (Sies, 1999). Elevated Cu or Cd concentrations in water can induce the overproduction of ROS in aquatic animals (Viarengo *et al*., 1990; Almeida *et al*., 2004; Company *et al*., 2004; Chandran *et al*., 2005; Upadhyay and Panda, 2010). These ROS can negatively affect DNA, RNA, ribosome synthesis, and inactivate enzyme systems (Stohs *et al*., 2000). Furthermore, it can cause peroxidation of cell membrane lipids

* Corresponding author. E-mail: wzhang@ouc.edu.cn

(Bagchi *et al*., 1996).

Reactive oxygen species is an inevitable part of aerobic life. It is a collective term for oxygen-centered radicals, such as superoxide, hydroxyl and non-radical oxygen derivatives, namely hydrogen peroxide and singlet oxygen (Scalbert *et al*., 2005). If these noxious oxygen derivatives are not controlled by antioxidative defense systems, oxidative stress caused by biological, physical and chemical stresses occurs (Sies, 1985). It is well known that, to protect against oxidative stress, all aerobic cells have developed antioxidative defense and redox balance systems (Wang *et al*., 2006). Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferaes (GST) are the major antioxidative enzymes, which acted as cellular catalysts in removing ROS. Glutathione (GSH) can be a substrate of GST and also participate in the conjugation and detoxification processes of pollutants. The induction of anti-oxidative enzymes or nonenzymatic antioxidatives (*e.g*., GSH) could serve as indices to study the impact of toxic chemical exposure on organisms (Verlecar *et al*., 2008).

Abalone *Haliotis discus hannai*, a large algivorous marine gastropod, is the most commercially important mollusk species cultured in China. It has a habitation style that they are capable of migrating for distances. Therefore, they could potentially exhibit different responses to the pollutants from the marine environment in different styles. Moreover, water quality tends to cause abalone population decline together with exploitation of fishing activities, deterioration of natural habitats and food availability (Gorski and Nugegoda, 2006). There are a few investigations about the toxicity effects of heavy metals on abalone. Tsai *et al*. (2004) found that the shell growth of *H. diversicolor supertexta* was greatly inhibited by increasing concentration of waterborne zinc (Zn) from 0.125 to 1.0 $\mu g\,mL^{-1}$. Huang *et al*. (2010) reported that waterborne Cd and silver (Ag) concentrations in the *H. diversicolor* both significantly increased after 7 weeks metals exposure. The purpose of this study was to analyze the effects of waterborne copper and cadmium on antioxidative responses, lipid peroxidation and heavy metals accumulation in abalone *H. discus hannai*.

2 Materials and Methods

2.1 Abalone

Healthy juvenile abalone *H. discus hannai* (initial body weight: $7.49\,g \pm 0.01\,g$) were collected from a spawning of the Laoshan Fisheries Farm, Qingdao, China. Prior to experiment, animals were acclimated to laboratory conditions for 2 weeks. They were fed with fresh kelp to satiation once daily at 18:00. The contents of Cu and Cd in kelp were 4.45 and $1.20\,mg\,kg^{-1}$, respectively, as determined by the flame atomic absorption spectrophotometry (AAS) (SOLAAR M6, Thermo, USA).

2.2 Experimental Design and Sample Collection

The exposure experiment was conducted in tanks (100 L) for 28 days. Abalone were divided into nine groups and subjected to nine treatments. These treatments were the control, four concentrations of waterborne Cu and four concentrations of waterborne Cd, respectively. Concentrations of Cu and Cd in the seawater used in the control group were $4.50\,\mu g\,L^{-1}$ and $0.45\,\mu g\,L^{-1}$, respectively, as determined by the AAS. The $CuSO_4{\cdot}5H_2O$ was added to the seawater to achieve 4 graded concentrations of waterborne Cu. They were twice ($0.02\,mg\,L^{-1}$), four times ($0.04\,mg\,L^{-1}$), six times ($0.06\,mg\,L^{-1}$) and eight times ($0.08\,mg\,L^{-1}$) of Cu concentration according to the 'water quality standard for fisheries in China (WQSFC)' ($Cu \leq 0.01\,mg\,L^{-1}$), respectively. The analyzed concentrations of waterborne Cu were $0.024 \pm 0.00\,mg\,L^{-1}$, $0.045 \pm 0.00\,mg\,L^{-1}$, $0.065 \pm 0.00\,mg\,L^{-1}$ and $0.082 \pm 0.00\,mg\,L^{-1}$, respectively. The $CdCl_2{\cdot}2.5H_2O$ was added to the seawater to achieve 4 graded concentrations of waterborne Cd. They were five times ($0.025\,mg\,L^{-1}$), ten times ($0.05\,mg\,L^{-1}$), fifty times ($0.25\,mg\,L^{-1}$) and one hundred times ($0.5\,mg\,L^{-1}$) of the Cd concentration according to the WQSFC ($Cd \leq 0.005\,mg\,L^{-1}$), respectively. The analyzed concentrations of waterborne Cd were 0.027 ± 0.00, 0.050 ± 0.00, 0.27 ± 0.01 and $0.53 \pm 0.02\,mg\,L^{-1}$, respectively. There were three replicates for each treatment, and each replicate (tank) consisted of 60 abalones. Animals were fed with the fresh kelp once daily at 18:00. Every morning, feces and uneaten kelp were removed to maintain the water quality. During the exposure period, the water temperature and salinity were 18–22℃ and 22–28, respectively, pH 7.4–7.9, and dissolved oxygen was not less than $6\,mg\,L^{-1}$. The abalone was exposed to a 12-h light: 12-h dark photoperiod regime. Half volume of the water was exchanged with fresh seawater twice daily.

Prior to the exposure experiment, eight abalones were sampled. During the exposure period, eight abalones per tank were sampled on the 1st, 3rd, 6th, 10th, 15th and 21st day, respectively. On the 28th day, all the live abalone was sampled. The mortality of abalone was recorded every day. The sampled abalone was not included into the calculation of mortality. Hepatopancreas and muscle were isolated, washed in cold saline (0.86% NaCl), and stored at −80℃. The sea water was sampled every two days during the experiment. Metal concentrations in hepatopancreas, muscle and seawater were measured by the method of AAS.

2.3 Anti-oxidative Enzyme Activity Assay

For assay of anti-oxidative enzymes activity in hepatopancreas, samples were homogenized in cold (4℃) 0.86% NaCl at a ratio of 1:10. A crude extract was obtained by centrifuging the homogenate at $1700 \times g$ for 10 min at 4℃. Supernatants were used for subsequent analysis.

Based on the dye-binding procedure, the total protein concentrations in supernatants were determined (Bradford 1976). Bovine serum albumin was used as the standard.

Activity of CAT was determined using a spectrophotometric assay of hydrogen peroxide, which based on the

formation of its stable complex with ammonium molydate at 405 nm (Goth, 1991). One unit of CAT activity was defined as the degradation of 1 μmol H_2O_2 per second per mg tissue protein.

Activity of SOD was determined through the inhibition of nitrobluetetrazolium reduction by O^{2-} generated by the xanthine/xanthine oxidase system (Huang *et al*., 2006). The optical density was measured at 550 nm. One SOD activity unit was defined as the enzyme amount causing 50% inhibition in 1 mL reaction solution per mg tissue protein. The result was expressed as U per mg protein.

Activity of GPx was assayed spectrophotometrically by measuring the decrease of the enzymatic reaction of glutathione at 412 nm (Li *et al*., 2005). One unit of GPx activity was defined as the decrease in the amount of 1 μmol L^{-1} glutathione in the enzymatic reaction system of 1 mg protein per min. The GPx activity was expressed as U per mg protein.

Activity of GST was determined using the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) with reduced glutathione (Habig *et al*., 1974). This activity had a positive linear relationship with the amount of reduced glutathione decreasing. Measurements were recorded at a wavelength of 412 nm. One unit of GST activity was defined as the decrease in the amount of 1 μmol L^{-1} glutathione in the enzymatic reaction system of 1 mg protein per min.

2.4 Glutathione (GSH) Contents

The reduced GSH content in hepatopancreas was determined as described by Anderson (1985). GSH is oxidized by 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) to give oxidized glutathione (GSSH) with stoichiometric formation of 5-thio-2-nitrobenzoic acid (TNB). GSSH is reduced to GSH by the action of the highly specific glutathione reductase (GSSH reductase) and NADPH. The rate of TNB formation is followed at 412 nm and is proportional to the sum of GSH and GSSH present. Glutathione was quantified using a standard curve of known concentration of GSH.

2.5 Lipid Peroxidation (LPO) Assay

Level of malondiadehyde (MDA) in hepatopancreas was measured using the thiobarbituric acid (TBA) fluorometric assay with 1, 1, 3, 3-teraethoxypropane as a standard (Şahin *et al*., 2007). Levels of MDA were determined fluorometrically with excitation and emission wavelengths of 532 nm and 547 nm, respectively. The LPO measurements were carried out with MAD detection kit (Nanjing Jiancheng Bioengineering Institute, China).

2.6 Statistical Analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2003. Data were analyzed by one-way analysis of variance (ANOVA). When significant differences ($P<0.05$) were found, means were compared using the Tukey's test. All the data were presented as means ± S.E. (standard error).

3 Results

3.1 Survival

The cumulative mortality of abalone after Cu exposure is shown in Fig.1. All abalone exposed to 0.02 mg L^{-1} of waterborne Cu and the control abalone were survival after the 28 days exposure. The first dead abalone exposed to 0.04, 0.06 and 0.08 mg L^{-1} waterborne Cu was found on the 7th day, 5th and 2nd day, respectively. The last dead abalone was found on the 21st, 10th and 6th day, respectively. All abalone exposed to waterborne Cd for 28 days survived.

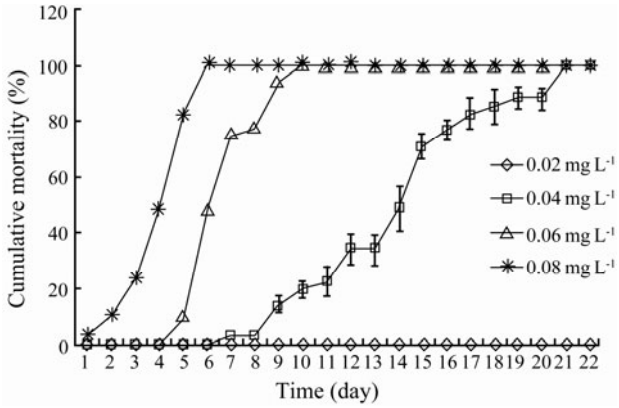

Fig.1 The cumulative mortality of *Haliotis discus hannai* exposed to waterborne Cu at concentrations of 0.02, 0.04, 0.06 and 0.08 mg L^{-1}.

3.2 Activities of Anti-oxidative Enzymes and Contents of GSH after Cu Exposure

The activities of SOD, CAT, GPx, GST and the content of GSH in the hepatopancreas of abalone in the Cu exposure trial are presented in Table 1. Generally, activities of SOD in hepatopancreas under all Cu concentrations followed a decreasing trend as the exposure time prolonged. On the 1st day, activities of SOD exposed to 0.04, 0.06 or 0.08 mg L^{-1} waterborne Cu were significantly decreased in comparison with that in the control ($P<0.05$).

In general, activities of CAT in all Cu exposure were higher than those in the control from day 1 to day 21. Moreover, activities of CAT in all Cu exposure were significantly higher than those in the control on the 1st and 3rd day ($P<0.05$). The highest values of CAT were found as 19.60 U mg^{-1} protein on day 1, 20.04 U mg^{-1} protein on day 1, 19.77 U mg^{-1} protein on day 3 and 20.35 U mg^{-1} protein on day 1 exposed to 0.02, 0.04, 0.06 and 0.08 mg L^{-1} waterborne Cu, respectively.

The GPx activities in all Cu exposure treatments were higher than those in the control on the 1st, 3rd, 6th, 10th and 15th day. The activity of GPx in the hepatopancreas of 0.06 mg L^{-1} and 0.08 mg L^{-1} Cu groups significantly increased from day 3 to day 10 and day 3 to day 6, respectively ($P<0.05$). The activity of GPx exposed to 0.02 mg L^{-1} and 0.04 mg L^{-1} Cu treatments were significantly increased from day 6 to day 15 and day 6 to day 21, respectively ($P<0.05$).

Table 1 Activities of SOD, CAT, GPx, GST and the content of GSH in hepatopancreases of *H. discus hannai* exposed to graded levels of waterborne Cu for 28 days

Enzymes	Dose ($mg L^{-1}$)	Day 0	Day 1	Day 3	Day 6	Day 10	Day 15	Day 21	Day 28
SOD	Control	29.66±0.55	30.12±1.26^{A}	28.74±0.39^{A}	28.53±0.44^{A}	27.82±1.15^{A}	27.82±1.15^{A}	27.38±1.36^{A}	29.69±0.82^{A}
	0.02	29.66±0.55	33.74±1.96^{A}	21.92±1.18^{BC}	29.52±0.71^{A}	19.38±1.68^{B}	19.38±1.68^{B}	19.34±1.01^{B}	17.63±0.81^{B}
	0.04	29.66±0.55^{a}	20.03±1.68^{Bab}	17.73±0.18^{Cab}	26.09±0.40^{Bab}	20.17±1.69^{Bab}	20.17±1.69^{Bab}	16.53±0.18^{Bb}	ND
	0.06	29.66±0.55^{a}	19.82±1.79^{Bb}	24.87±1.69^{ABab}	18.24±0.08^{Cab}	20.17±1.46^{Bab}	ND	ND	ND
	0.08	29.66±0.55^{a}	16.81±1.59^{Bb}	19.97±0.84^{Cb}	19.10±0.68^{Cab}	ND	ND	ND	ND
CAT	Control	10.78±0.40	10.81±0.66^{B}	10.66±1.18^{B}	11.95±0.13^{B}	10.47±0.87^{B}	9.52±0.25^{B}	9.86±0.30^{B}	11.33±0.82
	0.02	10.78±0.40^{bc}	19.60±2.32^{Aa}	18.90±1.32^{Aa}	17.08±0.83^{Aab}	15.10±0.08^{Aabc}	14.68±1.26^{Aabc}	13.61±1.33^{ABabc}	9.02±1.71^{c}
	0.04	10.78±0.40^{c}	20.04±0.42^{Aa}	16.50±1.27^{Aab}	16.98±0.88^{Aab}	12.79±1.37^{ABbc}	16.82±1.52^{Aab}	13.91±0.68^{Abc}	ND
	0.06	10.78±0.40^{b}	17.44±0.29^{Aa}	19.77±1.01^{Aa}	12.08±0.25^{Bb}	12.61±0.84^{ABb}	ND	ND	ND
	0.08	10.78±0.40^{b}	20.35±1.03^{Aa}	18.66±0.40^{Aa}	11.07±1.14^{Bb}	ND	ND	ND	ND
GPx	Control	50.75±2.58	53.11±2.67	46.46±4.60^{B}	47.95±1.67^{B}	48.66±2.63^{B}	49.05±1.27^{C}	58.7±4.56^{B}	52.13±4.19^{A}
	0.02	50.75±2.58^{bc}	58.75±5.73^{abc}	55.59±4.12^{ABabc}	69.20±4.41^{Aab}	74.24±8.55^{Aa}	69.85±1.42^{Bab}	41.38±0.43^{Cdc}	25.83±2.94^{dB}
	0.04	50.75±2.58^{c}	70.14±11.25^{bc}	55.59±1.44^{ABc}	62.82±2.15^{Abc}	86.53±6.18^{Ab}	114.86±7.45^{Aa}	70.96±1.26^{Abc}	ND
	0.06	50.75±2.58^{b}	74.76±6.91^{a}	64.14±1.06^{Aab}	62.80±1.07^{Aab}	74.70±2.48^{Aa}	ND	ND	ND
	0.08	50.75±2.58^{b}	77.70±6.03^{b}	60.23±1.49^{Aab}	62.37±2.92^{Aa}	ND	ND	ND	ND
GST	Control	48.38±0.55	57.01±3.37	51.63±1.18^{A}	48.73±1.99^{AB}	57.87±1.84^{A}	56.24±2.37^{A}	51.98±4.10^{A}	53.13±1.76^{A}
	0.02	48.38±0.55^{ab}	51.78±1.30^{a}	56.34±2.13^{Aa}	55.83±6.42^{Aa}	51.58±3.00^{Aa}	62.24±1.39^{Aa}	35.28±1.96^{Bbc}	27.98±1.63^{Bc}
	0.04	48.38±0.55^{ab}	47.67±1.73^{abc}	57.18±4.38^{Aa}	25.14±2.14^{Cd}	33.02±4.78^{Bd}	33.80±0.02^{Bcd}	36.36±2.96^{Bbcd}	ND
	0.06	48.38±0.55^{ab}	44.83±2.01^{b}	56.61±3.43^{Aa}	22.32±1.57^{Cc}	29.78±2.17^{Cc}	ND	ND	ND
	0.08	48.38±0.55^{ab}	56.81±4.97^{a}	33.55±6.13^{Bb}	33.38±4.73^{BCb}	ND	ND	ND	ND
GSH	Control	4.47±0.04	4.07±0.20^{B}	4.19±0.27^{B}	4.23±0.08^{B}	3.92±0.19^{B}	3.50±0.45^{B}	3.90±0.32^{B}	4.37±0.32
	0.02	4.47±0.04^{c}	7.11±0.37^{Aabc}	7.23±0.67^{Aab}	7.55±0.93^{Aa}	7.78±0.69^{Aa}	7.27±0.46^{Aab}	5.38±0.29^{Babc}	4.80±0.34^{bc}
	0.04	4.47±0.04^{c}	6.44±0.36^{Aabc}	5.64±0.39^{ABc}	5.49±0.23^{ABc}	5.95±0.46^{Abc}	8.05±0.25^{Aa}	7.75±0.77^{Aab}	ND
	0.06	4.47±0.04^{c}	6.85±0.21^{Aa}	7.35±0.47^{Aa}	5.39±0.36^{ABbc}	6.74±0.21^{Aab}	ND	ND	ND
	0.08	4.47±0.04^{b}	7.44±0.71^{Aa}	6.18±0.44^{ABab}	6.54±0.84^{ABab}	ND	ND	ND	ND

Notes: Values are expressed as mean ± standard error. CAT, catalase activity (U per mg Prot); SOD, superoxide dismutase (U per mg Prot); GPx, glutathione peroxidase (U per mg Prot); GST, glutathione S-transferases (U per mg Prot); GSH, glutathione (mg per g Prot). Different lowercase letters in rows indicated significant differences ($P<0.05$) as determined by Tukey's test. Different uppercase letters in column indicated significant differences ($P<0.05$) as determined by Tukey's test. ND: No data because all the abalone was dead.

In general, activities of GST in all Cu exposure treatments followed a decreasing pattern as time prolonged. Activities of GST were first significantly inhibited at day 3 ($P<0.05$). Activities of GST exposed to 0.02, 0.04, 0.06 and 0.08 $mg L^{-1}$ of Cu were significantly inhibited from day 21 to day 28, day 6 to day 21, day 6 to day 10 and day 3 to day 6, respectively ($P<0.05$).

The GSH contents in all Cu exposure treatments under all exposure time were higher than those in the control. The contents of GSH in all Cu exposure treatments were significantly higher than those in the control on the 1st, 10th and 15thday ($P<0.05$). The GSH contents were significantly higher than those in the control at day 3 exposed to 0.02 $mg L^{-1}$ and 0.06 $mg L^{-1}$ waterborne Cu exposure, at day 6 exposed to 0.02 $mg L^{-1}$ waterborne Cu exposure and at day 21 exposed to 0.04 $mg L^{-1}$ waterborne Cu exposure ($P<0.05$).

3.3 Activities of Anti-oxidative Enzymes and Contents of GSH after Cd Exposure

The activities of SOD, CAT, GPx, GST and the content of GSH in the hepatopancreas of abalone in the Cd exposure trail are presented in Table 2. Generally, activities of SOD exposed to 0.05, 0.25 and 0.5 $mg L^{-1}$ waterborne Cd followed a same pattern. They were firstly increased and then decreased to the control level and increased again during Cd exposure. The SOD activity was first significantly increased on the 6th day ($P<0.05$). There was no significant difference in SOD activity compared with those in the control on the 10th, 15thand 28th day ($P>0.05$).

In generally, activities of CAT in all waterborne Cd exposure treatments followed a same pattern. They were firstly increased and then decreased to the control level and increased again. The CAT activity was first significantly increased on the 6th day ($P<0.05$).

Activities of GPx were first significantly inhibited at day 6 ($P<0.05$). Activities of GPx exposed to 0.025 $mg L^{-1}$ and 0.05 $mg L^{-1}$ waterborne Cd were first significantly inhibited at day 10 ($P<0.05$). The activity of GPx in all waterborne Cd treatments was lower than those in the control from day 10 to day 21.

Activities of GST in all waterborne Cd exposure treatments followed a same pattern. They were firstly increased and then decreased to the control level and increased again. On the 3rd and 28th day, activities of GST in

Table 2 Activities of SOD, CAT, GPx, GST and the content of GSH in hepatopancreases of *H. discus hannai* exposed to graded levels of waterborne Cd for 28 days

Enzymes	Dose ($mg\,L^{-1}$)	Day 0	Day 1	Day 3	Day 6	Day 10	Day 15	Day 21	Day 28
SOD	Control	29.66±0.55	30.12±1.26^{AB}	28.74±0.39^{B}	28.53±0.44^{C}	27.82±1.15	29.42±0.29	27.38±1.36^{B}	29.69±0.82^{B}
	0.025	29.66±0.55^{ab}	27.65±1.34^{ABab}	33.74±2.30^{ABab}	35.59±1.12^{Aa}	28.36±3.68^{ab}	30.55±5.10^{ab}	22.11±1.31^{Cb}	29.88±1.37^{Bab}
	0.05	29.66±0.55^{cd}	32.21±1.16^{Abcd}	38.88±1.45^{Aa}	31.84±0.27^{Bcd}	28.61±0.84^{d}	33.53±1.17^{bc}	27.36±1.08^{Bd}	36.93±0.97^{Aab}
	0.25	29.66±0.55^{cd}	25.52±1.46^{Bd}	31.70±1.73^{Bbc}	33.86±0.05^{ABabc}	25.89±0.39^{d}	33.14±0.53^{abc}	36.44±0.72^{Aab}	37.38±1.27^{Aa}
	0.5	29.66±0.55^{bc}	26.01±1.65^{ABd}	31.03±0.06^{Bbc}	32.61±0.36^{Bb}	29.07±0.10^{cd}	32.45±0.32^{b}	36.92±0.02^{Aa}	36.18±0.60^{Aa}
CAT	Control	10.78±0.40	10.81±0.66	10.66±1.18	11.95±0.13^{D}	10.47±0.87^{B}	9.52±0.25^{AB}	9.86±0.30^{D}	11.33±0.82^{D}
	0.025	10.78±0.40^{c}	11.24±0.56^{c}	11.22±1.52^{c}	21.79±0.76^{Cb}	12.23±1.33^{Bc}	8.90±1.41^{ABc}	30.42±0.54^{Aa}	26.74±1.41^{Cab}
	0.05	10.78±0.40^{d}	9.67±0.74^{d}	9.34±0.18^{d}	31.67±0.69^{Ab}	21.82±1.58^{Ac}	12.33±1.52^{Ad}	33.27±0.80^{Ab}	43.09±0.87^{Aa}
	0.25	10.78±0.40^{d}	8.58±1.11^{d}	11.30±0.32^{c}	23.24±1.15^{BCb}	20.29±0.95^{Abc}	9.63±1.25^{ABd}	17.91±1.15^{Cc}	31.33±0.42^{Ba}
	0.5	10.78±0.40^{c}	9.27±0.56^{c}	10.56±1.52^{c}	26.34±0.76^{Bb}	22.86±1.33^{Ac}	6.80±1.41^{Bc}	26.01±0.54^{Ba}	35.15±0.63^{Bab}
GPx	Control	50.75±2.58	46.16±7.45	46.46±4.60	50.84±3.33^{A}	48.66±2.63^{A}	52.81±3.97^{A}	58.70±4.56^{A}	52.13±4.19^{AB}
	0.025	50.75±2.58^{ab}	47.57±0.18^{abc}	53.14±6.97^{ab}	59.11±2.07^{Aa}	30.06±0.73^{Bc}	42.35±2.67^{ABabc}	39.12±3.23^{Bbc}	46.80±5.87^{ABCabc}
	0.05	50.75±2.58^{ab}	45.59±0.41^{ab}	48.23±4.98^{ab}	48.53±0.47^{ABab}	29.52±1.92^{Bc}	27.64±0.97^{Cc}	40.17±3.44^{Bbc}	56.96±3.31^{Aa}
	0.25	50.75±2.58^{ab}	42.18±2.10^{bc}	61.86±3.80^{a}	37.86±3.16^{BCbc}	31.23±1.61^{Bc}	34.57±3.08^{BCc}	41.19±4.06^{ABbc}	33.26±0.17^{Cc}
	0.5	50.75±2.58^{a}	39.48±1.68^{abc}	49.60±4.30^{ab}	33.54±2.43^{Cc}	27.46±2.96^{Bc}	35.15±1.71^{BCbc}	35.72±3.37^{Bbc}	37.04±3.96^{BCabc}
GST	Control	48.38±0.55	57.01±3.37	56.13±1.18^{C}	55.19±0.34^{AB}	54.56±1.52	54.22±4.82	51.98±4.10	53.13±1.76^{B}
	0.025	48.38±0.55^{b}	55.20±1.99^{ab}	58.52±1.52^{BCab}	48.73±1.99^{Aab}	57.87±1.84^{ab}	56.24±2.37^{ab}	51.02±1.67^{ab}	60.11±3.20^{ABa}
	0.05	48.38±0.55^{dc}	57.18±4.07^{bc}	64.74±3.05^{Bb}	41.30±0.42^{Bd}	49.85±2.47^{cd}	52.23±1.12^{bcd}	48.79±2.46^{cd}	70.17±3.97^{Aa}
	0.25	48.38±0.55^{dc}	54.29±2.71^{dc}	78.09±3.01^{Aa}	41.25±3.46^{Bd}	53.22±3.71^{cd}	57.34±1.09^{bc}	58.01±1.34^{bc}	69.06±4.56^{Aab}
	0.5	48.38±0.55^{b}	49.65±3.18^{b}	61.46±3.41^{BCab}	50.12±0.77^{Ab}	55.77±4.74^{ab}	51.49±5.27^{bc}	54.52±0.24^{ab}	69.98±3.17^{Aa}
GSH	Control	4.47±0.04	4.07±0.20	4.19±0.27^{C}	4.23±0.08	3.92±0.19^{A}	3.50±0.45^{B}	3.90±0.32^{B}	4.37±0.32^{C}
	0.025	4.47±0.04^{ab}	3.81±0.12^{bc}	4.73±0.19^{BCa}	4.66±0.12^{a}	3.07±0.09^{Bcd}	2.77±0.32^{Bd}	2.46±0.15^{Cd}	2.70±0.13^{Cd}
	0.05	4.47±0.04^{b}	3.75±0.27^{bc}	5.72±0.06^{Aa}	4.49±0.19^{b}	2.81±0.16^{Bd}	2.99±0.13^{Bcd}	2.61±0.19^{Cd}	2.79±0.13^{Cd}
	0.25	4.47±0.04^{bc}	3.30±0.21^{c}	5.28±0.11^{ABb}	4.34±0.10^{bc}	3.88±0.24^{Abc}	4.38±0.28^{ABbc}	4.41±0.40^{Bbc}	7.13±0.59^{Ba}
	0.5	4.47±0.04^{cde}	3.31±0.16^{bc}	5.14±0.23^{ABe}	4.69±0.15^{cd}	3.97±0.10^{Ade}	5.60±0.51^{Ac}	8.78±0.14^{Ab}	10.63±0.65^{Aa}

Notes: Values are expressed as mean ± standard error. CAT, catalase activity (U per mg Prot); SOD, superoxide dismutase (U per mg Prot); GPx, glutathione peroxidase (U per mg Prot); GST, glutathione S-transferases (U per mg Prot); GSH, glutathione (mg per g Prot). Different lowercase letters in rows indicated significant differences ($P<0.05$) as determined by Tukey's test. Different uppercase letters in column indicated significant differences ($P<0.05$) as determined by Tukey's test.

all waterborne Cd treatments were higher than those in the control, and significantly increased at day 3 exposed to 0.05 $mg\,L^{-1}$ and 0.25 $mg\,L^{-1}$ waterborne Cd and at day 28 exposed to 0.05, 0.25 and 0.5 $mg\,L^{-1}$ waterborne Cd ($P<0.05$). However, activities of GST in all waterborne Cd treatments showed no significant difference with those in the control from day 10 to day 21 ($P>0.05$).

Generally, the GSH contents in all waterborne Cd treatments firstly increased and then decreased to control level, and then those exposed to 0.025 $mg\,L^{-1}$ and 0.05 $mg\,L^{-1}$ waterborne Cd were significantly decreased ($P<0.05$), and those exposed to 0.25 $mg\,L^{-1}$ and 0.5 $mg\,L^{-1}$ waterborne Cd were significantly increased ($P<0.05$).

3.4 Lipid Peroxidation in Hepatopancreas

Lipid peroxidation was expressed as the MDA contents in the hepatopancreas. The MDA contents in the hepatopancreas in Cu and Cd exposure trial are presented in Table 3.

Contents of MDA in all Cu treatments followed a same pattern. They increased first and then decreased to the control level. The MDA level of 0.02 $mg\,L^{-1}$ Cu treatment was significantly increased at day 3 and day 6 ($P<0.05$), and decreased to the control level from day 10 to day 28. The MDA level of 0.04 $mg\,L^{-1}$ Cu treatment was significantly increased at day 6 ($P<0.05$) and was higher than those in the control from day 10 to day 21 in a whole. However, the MDA levels of 0.06 $mg\,L^{-1}$ or 0.08 $mg\,L^{-1}$ Cu treatments under the exposure days were higher than those in the control. The MDA level did not significantly change under all Cd concentrations for 28 days compared with those in the control ($P>0.05$).

3.5 Metal Concentrations in Hepatopancreas and Muscle

The Cu and Cd accumulation in hepatopancreas and muscles of *H. discus hannai* is present in Table 4 and Table 5. In generally, the metals accumulation in both hepatopancreas and muscles of abalone increased with waterborne metals concentration and exposure time. Moreover, hepatopancreas accumulated more Cu or Cd than muscle. The maximum content of Cu in hepatopancreas and muscles were 81.56 $\mu g\,g^{-1}$ wt and 24.57 $\mu g\,g^{-1}$ wt both exposed to 0.02 $mg\,L^{-1}$ at day 21, which increased by 10 and 5 times compared with those in the control, respectively. The maximum content of Cd in hepatopancreas and muscles were 282.96 $\mu g\,g^{-1}$ wt and 10.40 $\mu g\,g^{-1}$ wt, which

increased by 70 times and 10000 times compared with those in the control, respectively.

Table 3 The content of MDA in hepatopancreases of *H. discus hannai* exposed to graded levels of waterborne Cu or Cd for 28 days

MDA	Dose ($mg L^{-1}$)	Day 0	Day 1	Day 3	Day 6	Day 10	Day 15	Day 21	Day 28
Cu	Control	4.93±0.12	5.23±0.20	$5.56±0.46^{B}$	$5.23±0.20^{C}$	$4.62±0.26^{AB}$	$5.58±0.11^{AB}$	$4.73±0.40^{AB}$	6.23±0.38
	0.02	$4.93±0.12^{bc}$	$6.59±0.65^{bc}$	$9.37±0.70^{Aa}$	$6.59±0.65^{ABab}$	$6.40±0.33^{Abc}$	$4.78±0.56^{Bc}$	$4.44±0.44^{Bc}$	$4.96±0.67^{bc}$
	0.04	$4.93±0.12^{cd}$	$6.38±0.09^{bc}$	$6.60±0.68^{Bbc}$	$8.70±0.02^{Aa}$	$4.47±0.56^{Bd}$	$7.01±0.24^{Aab}$	$5.97±0.08^{Abcd}$	ND
	0.06	$4.93±0.12^{b}$	$5.91±0.03^{ab}$	$6.83±0.54^{Ba}$	$5.91±0.03^{BCab}$	$5.95±0.40^{ABab}$	ND	ND	ND
	0.08	$4.93±0.12^{b}$	$6.21±0.54^{ab}$	$7.16±0.17^{ABa}$	$6.74±0.12^{BCa}$	ND	ND	ND	ND
Cd	Control	4.93±0.12	5.92±0.31	5.56±0.46	4.99±0.49	4.62±0.26	5.58±0.11	4.73±0.40	6.23±0.38
	0.025	4.93±0.12	5.23±0.20	5.45±1.01	8.79±1.08	4.95±0.57	5.70±1.03	6.68±0.26	6.92±0.77
	0.05	4.93±0.12	4.45±0.44	6.50±1.00	5.10±0.85	4.53±0.42	5.56±0.86	5.94±0.19	8.36±1.18
	0.25	4.93±0.12	3.73±0.37	5.33±1.18	5.12±0.57	4.65±0.68	6.40±0.42	7.63±1.20	7.20±0.85
	0.5	4.93±0.12	4.26±0.43	5.92±0.15	6.02±0.13	4.35±0.26	5.36±0.26	6.36±0.98	5.84±0.27

Notes: Values are expressed as mean ± standard error. MDA, malondiadehyde (nmol per mg Prot). Different lowercase letters in rows indicated significant differences ($P<0.05$) as determined by Tukey's test. Different uppercase letters in column indicated significant differences ($P<0.05$) as determined by Tukey's test. ND: No data because all the abalone was dead.

Table 4 Concentrations of Cu in hepatopancreas and muscle of *H. discus hannai* exposed to graded levels of waterborne Cu for 28 days

Tissues ($\mu g g^{-1}$)	Dose ($mg L^{-1}$)	Day 0	Day 1	Day 3	Day 6	Day 10	Day 15	Day 21	Day 28
Hepatopancreas	Control	8.09±0.63	8.40±0.86	$9.31±0.01^{C}$	$8.51±0.34^{C}$	$8.65±0.44^{Cd}$	$7.77±0.81^{C}$	$8.31±0.43^{C}$	$7.72±0.51^{B}$
	0.02	$8.09±0.63^{e}$	$11.98±0.96^{de}$	$14.33±0.95^{BCcd}$	$15.71±1.50^{BCcd}$	$20.27±0.54^{BCc}$	$24.20±1.29^{Bb}$	$24.63±1.94^{Bb}$	$38.12±1.42^{Aa}$
	0.04	$8.09±0.63^{f}$	$14.37±1.99^{ef}$	$18.77±1.24^{ABe}$	$31.56±1.62^{ABd}$	$39.03±1.18^{ABc}$	$68.38±1.74^{Ab}$	$81.56±1.58^{Aa}$	ND
	0.06	$8.09±0.63^{c}$	$12.49±1.41^{bc}$	$24.63±2.26^{Abc}$	$30.66±9.55^{ABb}$	$66.97±3.77^{Aa}$	ND	ND	ND
	0.08	$8.09±0.63^{c}$	$18.73±4.54^{bc}$	$24.56±2.90^{ABb}$	$39.02±2.48^{Aa}$	ND	ND	ND	ND
Muscle	Control	5.88±0.60	$5.29±0.25^{B}$	$5.84±0.20^{B}$	$5.14±0.37^{B}$	$5.64±0.21^{C}$	$4.93±0.34^{C}$	$4.74±0.46^{B}$	$5.47±0.37^{B}$
	0.02	$5.88±0.60^{d}$	$7.97±0.70^{ABCcd}$	$9.77±1.53^{ABbcd}$	$8.39±0.70^{Bbcd}$	$13.14±0.94^{Bb}$	$10.73±0.84^{Bbc}$	$12.62±0.43^{Bbc}$	$14.96±0.90^{Aa}$
	0.04	$5.88±0.60^{d}$	$8.96±0.21^{Ad}$	$13.95±0.86^{Acd}$	$13.67±0.75^{Abcd}$	$20.74±1.21^{Aab}$	$17.08±0.72^{Aabc}$	$24.57±3.17^{Aa}$	ND
	0.06	$5.88±0.60^{c}$	$6.25±0.50^{ABc}$	$13.93±0.28^{Ab}$	$15.67±0.61^{Aab}$	$21.63±0.73^{Aa}$	ND	ND	ND
	0.08	$5.88±0.60^{c}$	$8.51±0.95^{Abc}$	$13.32±1.06^{Aab}$	$18.18±2.09^{Aa}$	ND	ND	ND	ND

Notes: Values are expressed as mean ± standard error. Different lowercase letters in rows indicated significant differences ($P<0.05$) as determined by Tukey's test. Different uppercase letters in column indicated significant differences ($P<0.05$) as determined by Tukey's test. ND: No data because all the abalone was dead.

Table 5 Concentration of Cd in hepatopancreas and muscle of *H. discus hannai* exposed to graded levels of waterborne Cd for 28 days

Tissues ($\mu g g^{-1}$)	Dose ($mg L^{-1}$)	Day 0	Day 1	Day 3	Day 6	Day 10	Day 15	Day 21	Day 28
Hepatopancreas	Control	2.76±0.19	$2.83±0.48^{D}$	$2.42±0.23^{D}$	$2.75±0.13^{C}$	$2.99±0.20^{D}$	$3.65±0.59^{D}$	$3.09±0.27^{C}$	$4.07±0.43^{E}$
	0.025	$2.76±0.19^{g}$	$2.23±0.14^{CDg}$	$4.05±0.25^{CDf}$	$5.86±0.25^{Ce}$	$7.37±0.12^{DCd}$	$10.23±0.11^{CDc}$	$14.14±0.03^{Cb}$	$21.13±0.22^{Da}$
	0.05	$2.76±0.19^{f}$	$4.29±0.04^{Cf}$	$6.42±0.67^{Cef}$	$10.09±0.39^{Cde}$	$14.17±1.10^{Ccd}$	$19.73±1.27^{Cc}$	$30.91±2.29^{Cb}$	$39.42±1.48^{Ca}$
	0.25	$2.76±0.19^{e}$	$8.89±0.53^{Be}$	$17.85±0.89^{Be}$	$42.24±2.72^{Bd}$	$60.77±0.72^{Bc}$	$63.61±7.07^{Bc}$	$136.16±2.40^{Bb}$	$151.46±3.57^{Ba}$
	0.5	$2.76±0.19^{e}$	$15.51±0.21^{Ae}$	$29.07±1.53^{Ae}$	$90.34±3.35^{Ad}$	$128.35±1.15^{Ac}$	$196.65±11.31^{Ab}$	$252.37±16.62^{Aab}$	$282.96±4.95^{Aa}$
Muscle	Control	$0.00±0.00^{b}$	$0.00±0.00^{Cb}$	$0.00±0.00^{Cb}$	$0.00±0.00^{Bb}$	$0.00±0.00^{Db}$	$0.00±0.00^{Cb}$	$0.00±0.00^{Cb}$	$0.00±0.00^{Ea}$
	0.025	$0.00±0.00^{c}$	$0.06±0.00^{Cc}$	$0.04±0.00^{Cc}$	$0.09±0.01^{Bc}$	$0.19±0.04^{Dc}$	$0.48±0.02^{Cb}$	$0.46±0.01^{Cb}$	$0.71±0.10^{Da}$
	0.05	$0.00±0.00^{d}$	$0.11±0.01^{Cd}$	$0.11±0.01^{Cd}$	$0.33±0.06^{Bcd}$	$0.85±0.00^{Cbc}$	$0.75±0.07^{Cb}$	$1.59±0.26^{Ca}$	$1.59±0.13^{Ca}$
	0.25	$0.00±0.00^{d}$	$0.61±0.01^{Bcd}$	$0.62±0.08^{Bcd}$	$1.74±0.04^{Ac}$	$3.16±0.17^{Bb}$	$4.27±0.60^{Bab}$	$4.89±0.36^{Ba}$	$5.50±0.14^{Ba}$
	0.5	$0.00±0.00^{f}$	$0.81±0.05^{Ae}$	$0.97±0.06^{Ae}$	$2.29±0.26^{Ad}$	$4.18±0.11^{Ac}$	$8.06±0.06^{Ab}$	$9.97±0.18^{Ab}$	$10.40±0.17^{Aa}$

Notes: Values are expressed as mean ± standard error. Different lowercase letters in rows indicated significant differences ($P<0.05$) as determined by Tukey's test. Different uppercase letters in column indicated significant differences ($P<0.05$) as determined by Tukey's test.

4 Discussion

4.1 Impact of Waterborne Cu and Cd on Anti-oxidative Defense

Exposure to Cu or Cd has been shown to increase the formation of ROS and promote oxidative stress in mollucs (Company *et al*., 2004; Tamás *et al.*, 2009). Activities of anti-oxidative enzymes (*e.g*., SOD, CAT, GPx and GST) could be changed correspondingly to detoxify and clear ROS, and thus prevent aquatic animals from oxidative damages (Reméo *et al*., 2000; Asagba *et al*., 2008; Cao *et al*., 2010). During exposure to Cu or Cd, anti-oxidative enzymes activity and GSH content in the hepa-

topancreas continuously changed. It could be a resisting mechanism to the increase of ROS in hepatopancreas. Previous studies and this study suggested that antioxidants including anti-oxidative enzymes and GSH played an important role in preventing the hazardous effects of Cu or Cd, as they could be warning signals for severe damage to aquatic environment and the organisms living in it. In the present study, the first significant change of anti-oxidative enzymes activity or GSH content in hepatopancreas under Cu exposure was found on day 1 for SOD, CAT and GSH, on day 3 for GPx and GST, respectively (Table 1). However, the first significant change for Cd exposure was found on day 1 for SOD, day 3 for GST and GSH, day 6 for CAT and GPx, respectively (Table 2). Although the activities of anti-oxidative enzymes and content of GSH in the hepatopancreas of abalone were generally elevated, they did not follow the same pattern to resist the oxidative stress induced by Cu or Cd.

Superoxide dismutase catalyzes the transformation of superoxide radicals to H_2O_2 and O_2. It is the first enzyme to deal with oxyradicals (Kappus, 1985; Ruas *et al*., 2008). Catalase is a major anti-oxidative defense component that works primarily to catalyze the decomposition of H_2O_2 to H_2O, sharing this function with GPx. In the present study, SOD activity in hepatopancreas under all waterborne Cu concentrations followed a decreasing trend during 28-day exposure (Table 1). Reduction of SOD activity was also observed in the gill of mussels *Bathymodiolus azoricus* exposed to 25.6 μg L^{-1} Cu for 24 h (Company *et al*., 2004) and *Mytilus galloprovincialis* exposed to 5–25 μg L^{-1} Cu for 7 days (Maria and Bebianno, 2011). Jiang *et al*. (2011) also found a significant inhibitory effect of 0.6–7.2 mg L^{-1} Cu on the activity of SOD in hepatopancreas of juvenile Jian carp *Cyprinus carpio*var. However, under Cu exposure, CAT activity in hepatopancreas significantly increased at first and then decreased to the normal level as that in the control. Activity of GPx also significantly increased for most of Cu exposure (Table 1). It was suggested that stimulation of CAT and GPx activity could be a compensation for the decrease of SOD activity. On the other hand, the variation of CAT activity maybe indicate that CAT is gradually losing the capacity to eliminate the over production of ROS.

From the present data, however, it was suggested that the anti-oxidation defense mechanism in abalone for Cd stress was different from that for Cu stress. The reason was that the changes of SOD and CAT activities almost followed the same pattern, regardless of the Cd concentrations in water. Activities of SOD and CAT were stimulated first and then decreased to the normal level and elevated again (Table 2). However, Cd had a dose-dependent and time-dependant inhibition effect on GPx activity (Table 2). In previous studies, it was suggested that the response of SOD to the oxyradicals generated by Cd could minimize the harm of oxidation (Palace and Klaverkamp 1993; Company *et al*., 2004). At the same time, it was commonly assumed that any significant increase in SOD must be accompanied by a comparable increase in CAT and/or GPx activities (Warner, 1994). In the present study, it was true for CAT, but not for GPx. According to Yu (1994), in the presence of low H_2O_2 levels, organic peroxides are the preferred substrate for GPx, but at high H_2O_2 concentrations, they metabolized by CAT. It was suggested that exposure to waterborne Cd could induce high level of superoxide radical, which was transformed into H_2O_2 and O_2 by SOD. High levels of H_2O_2 stimulated the activity of CAT. For abalone in the present study, it was assumed that CAT and SOD were the dominant enzymes to eliminate ROS induced by Cd. Further study is needed to elucidate the difference in anti-oxidation response of abalone exposed to Cu and Cd.

GST activity in hepatopancreas of abalone under all the Cu concentrations followed a decreasing trend and was significantly inhibited by Cu with a clear concentration-response relationship (Table 1). According to Cunha *et al*. (2007), a significant *in vivo* reduction of marine gastropods *Nucella lapillus* GST activity by Cu was observed (47.6% at the highest concentration tested). A similar inhibition (about 44.4%) was observed in aquatic worms *Tubifex tubifex* after a 7-day exposure to 50–200 μg L^{-1} of Cu (Mosleh *et al*., 2005) and in the carp *Cyprinus carpio* after 96-h of exposure to 100 and 250 μg L^{-1} of Cu (Dautrememepuits *et al*., 2002). Contrary to Cu, the GST activity in hepatopancreas of abalone under Cd exposure was significantly induced at day 3 and day 28 (Table 2). In the previous studies, the same results were reported that GST activity was stimulated by Cd (Almeida *et al*., 2002; Basha and Rani, 2003; Giguere *et al*., 2005; Bouraoui *et al*., 2008). Fernández *et al*. (2010) found that the levels of Cd from Cartagena, Portman and Columbretes Islands were significantly higher than those at the remaining sampling sites. Correspondingly, mussels from these islands showed higher GST activity. In the present study, GST activity was significantly inhibited by Cu and significantly stimulated by Cd (Table1 and Table 2). It was suggested that GST might be more actively involved in detoxifying the toxicity of Cd than Cu.

Al-Subiai *et al*. (2009) found that GSH increased in the adductor muscle of *Mytlius edulis* exposed to Cu (40 μg L^{-1}) for 5 days. Meanwhile, increased levels of hepatic GSH had been reported in the striped mullet *Mugil cephalus* after 10 days acute exposure to Cd (10 mg L^{-1}) (Thomas and Wofford, 1984). Similar results of increasing hepatic GSH level were found in fish species such as rainbow trout, Nile tilapia, mullet and Atlantic croaker (Thomas *et al*., 1982; Thomas and Juedes, 1992; Tort *et al*., 1996; Firat *et al*., 2009). Generally, in the present study, GSH contents in hepatopancreas were increased by waterborne Cu or Cd exposure. The increasing GSH contents might be a mechanism to protect the abalone from oxidative stress or to perform detoxification.

4.2 Impact of Waterborne Cu and Cd on Lipid Peroxidation

Malondiadehyde (MDA) is the major reactive aldehyde resulting from the peroxidation of membrane polyunsatu-

rated fatty acid (PUFA) (Ohkawa *et al*., 1979). Copper ions can induce the production of ROS through a Fenton-like redox cycling mechanism (Halliwell and Gutteridge, 1984) and participate in the initiation and propagation of lipid peroxidation (Viarengo *et al*., 1990). In the present study, MDA was significantly increased in the hepatopancreas of abalone on day 3 and day 6 under 0.02 mg L^{-1}, and on day 6 under 0.04 mg L^{-1} Cu exposure (Table 3). Increasing MDA level induced by Cu is widely found in aquatic animals. Cu exposure caused an increase in lipid peroxidation in the liver of an estuarine fish *Pomatoschistus microps* (Vieira *et al*., 2009). Chelomin and Belcheva (1991) demonstrated that Cu accumulation in hepatopancreas cells was accompanied by a significant increase in MDA contents in scallop *Mizuho pectin yessoensis*. The MDA content increased significantly in the hepatopancreas of freshwater crab, *Oziotelphusa senex senex* exposed to 100 μg L^{-1} of Cu for 7 days (Reddy and Bhagyalakshmi, 1994). However, in the present study, MDA level in the hepatopancreas of abalone significantly decreased after 15-day exposure to 0.02 mg L^{-1} Cu (Table 3). It could be the results of anti-oxidation in abalone. And it could be the reason for abalone in this treatment to survive after 28-day waterborne Cu exposure. Concentrations of Cu in water more than 0.04 mg L^{-1} could be too high for abalone to survive due to the lipid peroxidation. Further study is needed to confirm it.

An elevation of lipid peroxidation has been observed in hepatic tissue of Atlantic croaker *Micropogonias undulates* exposed to 5 mg L^{-1} Cd for 33 days (Thomas and Wofford, 1993). However, in the present study, the MDA levels did not significantly change under all the Cd concentrations for 28 days (Table 3). According to Reddy and Bhagyalakshmi (1994), the levels of MDA did not significantly change in the hepatopancreas of *O. senex senex* exposed to 100 μg L^{-1} of Cd. Similar results was also found in crab *Scylla serrata* (Reddy, 1997) and scallop *Mizuhopecten yessoensis* (Chelomin and Belcheva, 1992). Vincent *et al*. (1989) reported that free radicals were involved at the early stages of Cd intoxication, and lipid peroxidation is primarily an outcome of generation of free radicals. However, the mechanism of Cd-induced lipid peroxidation is still not fully clarified. It is possible that the free radicals initiated by Cd were eliminated by anti-oxidation defense system in abalone, which significantly changed under Cd exposure.

4.3 Concentration of Metals in Hepatopancreas and Muscles

Copper (Cu) and Cd are the main pollution-causing metals (Yuan *et al*., 2004; Bopp *et al*., 2008; Meng *et al*., 2008; Yu *et al*., 2008). Mollusks can accumulate high concentrations of heavy metals and serve as bioindicators of metal contamination in the marine environment (Langston *et al*., 1998). The present study showed that Cu or Cd accumulation in the hepatopancreas and muscle followed a positive linear relationship with metal exposure concentration and exposure time in a whole (Table 4 and Table 5). Due to none survival abalone, the Cu stress trials in the present study were terminated on the 6th , 10th and 21st days with 0.08, 0.06 and 0.04 mg L^{-1} of waterborne Cu, respectively (Fig.1). However, the Cu accumulation in the hepatopancreas and muscle exposed to 0.06 mg L^{-1} Cu for 10 days or to 0.04 mg L^{-1} Cu for 21 days was higher than that of abalone exposed to 0.08 mg L^{-1} Cu for 6 days (Table 4). In other words, a slower rate of accumulation could result in a higher Cu concentration, and Cu did not accumulate to a critical body residue to lead to the mortality of abalone. Cadmium accumulation increased along with Cd concentrations and the exposure time. However, it had no significant effect on the survival of abalone. In the present study, it was suggested that tissue Cu or Cd accumulation had no significant correlation with the survival of abalone.

4.4 Mortality

In the present study, the maximum Cd concentration (0.5 mg L^{-1}), which was 100 times of China water quality standard for fisheries (WQSFC, Cd ≤ 0.005 mg L^{-1}), did not result in the death of abalone during 28 days exposure. However, waterborne Cu with the concentration of 4 times (0.04 mg L^{-1}), 6 times (0.06 mg L^{-1}) and 8 times (0.08 mg L^{-1}) of WQSFC caused 100% death of abalone on the 21st, 10th and 6th day, respectively. It was suggested that Cu is more toxic than Cd for abalone. In other words, abalone was more sensitive to waterborne Cu. Cheung *et al*. (2002) reported that the 96 h LC50 values of gastropod *Nassarius festivus* for Cu and Cd were 0.36 mg L^{-1} and 1.52 mg L^{-1}, respectively. It was also suggested that Cu was more toxic than Cd. Similar results were found in other marine gastropods, such as *Morula granulata* (Devi, 1997) and *Nassarius reticulates* (Kaland *et al*., 1992). Wang *et al*. (2007b) and Wang *et al*. (2007a) had found that juvenile mollusc were more sensitive to acute or chronic copper exposure than the tested organisms including cladocerans *Daphnia magna* and *Ceriodaphnia dubia*, an amphipod *Hyalella azteca*, fathead minnow *Pimephales promelas*, and rainbow trout *Oncorhynchus mykiss*. Meanwhile, mussels *Lampsilis siliquoidea* chronically exposed to 2 and 12 μg L^{-1} Cu showed significantly higher mortality (20.9%, 69.9% and 12.5%, respectively) than those in the control (Jorge *et al*., 2013). Although mollusks are more sensitive to Cu than Cd, very little is known about the actual reason. In the present study, anti-oxidant enzymes and GSH in the Cu experiment were significantly increased or decreased under most of the exposure time (Table 1). However, in the Cd experiment they almost followed a process which increased or decreased first and then changed to the control level (Table 2). It was suggested that Cu resulted in severer oxidative stress on anti-oxidant defense system than Cd. So the probable reason was that oxidative stress induced by Cu exceeded the capability of anti-oxidation defense system in abalone, and then the lipid peroxidation and even the mortality of abalone were significantly induced.

5 Conclusion

Exposure of abalone to waterborne Cd even at high concentrations did not cause significant lipid peroxidation, but waterborne Cu did. The anti-oxidative mechanism in the hepatopancreas of abalone to resist waterborne Cu did not follow the same pattern as that for waterborne Cd. Combined with the data on the cumulative mortalities, it was suggested that waterborne Cu was more toxic than Cd to abalone. The Cu or Cd accumulation in the hepatopancreas and muscle was a combined effect of waterborne metals concentration and exposure time. It did not directly relate to the survival of abalone.

Acknowledgements

This research was financially supported by grant from the National Natural Science Foundation of China (No. 30972262).

References

Almeida, E. A., Miyamoto, S., Bainy, A. C. D., Medeiros, M. H., and Mascio, P., 2004. Protective effect of phospholipid hydroperoxide glutathione peroxidase (PHGPx) against lipid peroxidation in mussels *Pernaperna* exposed to different metals. *Marine Pollution Bulletin*, **49**: 386-392.

Almeida, J. A., Diniz, Y. S., Marques, S. F. G., Faine, L. A., Ribas, B. O., Burneiko, R. C., and Novelli, E. L. B., 2002. The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to *in vivo* Cd contamination. *Environment International*, **27**: 673-679.

Al-Subiai, S. N., Jha, A. N., and Moody, A. J., 2009. Contamination of bivalve haemolymph samples by adductor muscle components: Implications for biomarker studies. *Ecotoxicology*, **18**: 334-342.

Anderson, M. E., 1985. Determination of glutathione and glutathione disulfide in biological samples. *Methods in Enzymology*, **113**: 548-555.

Asagba, S. O., Eriyamremu, G. E., and Igberaese, M. E., 2008. Bioaccumulation of cadmium and its biochemical effect on selected tissues of the catfish (*Clarias gariepinus*). *Fish Physiology and Biochemistry*, **34**: 61-69.

Bagchi, D., Bacghi, M., Hassoun, E. A., and Stohs, S. J., 1996. Cadmium induced excretion of urinary lipid metabolites, DNA damage, glutathione depletion and hepatic lipid peroxidation in Sprague-Dawley rats. *Biological Trace Element Research*, **52**: 143-154.

Basha, P. S., and Rani, A. U., 2003. Cadmium-induced antioxidant defense mechanismin freshwater teleost *Oreochromis mossambicus* (Tilapia). *Ecotoxicology Environmental Safety*, **56**: 218-221.

Bopp, S. F., Abicht, H. K., and Knauer, K., 2008. Copper-induced oxidative stress in rainbow trout gill cells. *Aquatic Toxicology*, **86**: 197-204.

Bouraoui, Z., Banni, M., Ghedira, J., Clerandeau, C., Guerbej, H., Narbonne, J. F., and Boussetta, H., 2008. Acute effects of cadmium on liver phase I and phase II enzymes and metallothionein accumulation on sea bream Sparusaurata. *Fish Physiology Biochemistry*, **34**: 201-207.

Bradford, M. M., 1976. A rapid method for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**: 248-254.

Cao, L., Huang, W., Liu, J., Yin, X., and Dou, S., 2010. Accumulation and oxidative stress biomarkers in Japanese flounder larvae and juveniles under chronic cadmium exposure. *Comparative Biochemistry Physiology (C)*, **151**: 386-392.

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*. *Comparative Biochemistry Physiology (C)*, **140**: 422-426.

Chelomin, V. P., and Belcheva, N. N., 1991. Alterations of microsomal lipid synthesis in gill cells of bivalve mollusk *Mizuhopecten yessoensis*in response to cadmium accumulation. *Comparative Biochemistry Physiology (C)*, **99**: 1-5.

Chelomin, V. P., and Belcheva, N. N., 1992. The effect of heavy metals on processes of lipid peroxidation in microsomal membranes from the hepatopancreas of bivalve mollusk *Mizuhopectin yessoensis*. *Comparative Biochemistry Physiology (C)*, **103**: 419-422.

Chen, J. L., Liu, W. X., Liu, S. Z., Lin, X. M., and Tao, S., 2004. An evaluation on heavy metal contamination in the surface sediments in Bohai Sea. *Marine Sciences*, **28**: 16-21.

Cheung, C. C. C., Zheng, G. J., Lam, P. K. S., and Richardson, B. J., 2002. Relationships between tissue concentrations of chlorinated hydrocarbons (polychlorinated biphenyls and chlorinated pesticides) and antioxidative responses of marine mussels, *Perna viridis*. *Marine Pollution Bulletin*, **45**: 181-191.

Company, R., Serafim, A., Bebianno, M. J., Cosson, R., Shillito, B., and Fiala-Médioni, A., 2004. Effect of cadmium, copper and mercury on antioxidant enzyme activities and lipid peroxidation in the gills of the hydrothermal vent mussel *Bathymodiolus azoricus*. *Marine Environmental Research*, **58**: 377-381.

Cunha, I., Mangas-Ramirez, E., and Guilhermino, L., 2007. Effects of copper and cadmium on cholinesterase and glutathione S-transferase activities of two marine gastropods (*Monodonta lineata* and *Nucella lapillus*). *Comparative Biochemistry Physiology (C)*, **145**: 648-657.

Dautrememepuits, C., Betoulle, S., and Vernet, G., 2002. Antioxidant response modulated by copper in healthy and parasitized carp (*Cyprinus carpio* L.) by *Ptychobothrium* sp. (Cestoda). *Biochimica et Biophysica Acta*, **1573**: 4-8.

De la Torre, F. R., Salibián, A., and Ferrari, L., 2000. Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium. *Environmental Pollution*, **109**: 277-282.

Devi, V. U., 1997. Heavy metal toxicity to an intertidal gastropod *Morula granulata* (Duclos): Tolerance to copper, mercury, cadmium and zinc. *Journal Environment Biology*, **18**: 287-290.

Fernández, B., Campillo, J. A., Martínez-Gómez, C., and Benedicto, J., 2010. Antioxidant responses in gills of mussel (*Mytilus galloprovincialis*) as biomarkers of environmental stress along the Spanish Mediterranean coast. *Aquatic Toxicology*, **99**: 186-197

Firat, O., Cogun, H. Y., Aslanyavrusu, S., and Kargin, F., 2009. Antioxidant responses and metal accumulation in tissues of Nile tilapia *Oreochromis niloticus* under Zn, Cd and Zn+Cd exposures. *Journal of Applied Toxicology*, **29**: 295-301.

Franco, R., Sanchez-Olea, R., Reyes-Reyes, E. M., and Panayiotidis, M. I., 2009. Environmental toxicity, oxidative stress and apoptosis: Ménage à trois. *Mutation Research*, **674**: 3-22.

Giguere, A., Peter, G. C., Campbell, L. H., and Cosu-Leguille, C., 2005. Metal bioaccumulation and oxidative stress in yel-

low perch (*Perca flavescens*) collected from eight lakes along a metal contamination gradient (Cd, Cu, Zn, Ni). *Canadian Journal Fisheries and Aquatic Sciences*, **62**: 563-577.

Goering, P. I., Waalkes, M. P., and Klassen, C. D., 1995. Toxicology of cadmium. In: *Toxicology of Metal Biochemical Aspects: Handbook of Experimental Pharmacology*. Goyer, R. A., and Cherian, M. G., eds., Springer-Verlag, New York, 189-213.

Gorski, J., and Nugegoda, D., 2006. Toxicity of trace metals to juvenile abalone, *Haliotis rubra* following short-term exposure. *Bulletin Environmental Contamination and Toxicology*, **77**: 732-740.

Goth, L., 1991. A simple method for determination of serum catalase activity and revision of reference range. *Clinica Chimica Acta*, **196**: 143-151.

Habig, W. H., Pabst, M. J., and Jakoby, W. B., 1974. Glutathione *S*-transferases. The first step in mercapturic acid formation. *The Journal of Biological Chemistry*, **249**: 7130-7139.

Halliwell, B., and Gutteridge, M. C., 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal*, **219**: 1-14.

Handy, R. D., 2003. Chronic effects of copper exposure versus endocrine toxicity: Two sides of the same toxicological process? *Comparative Biochemistry Physiology (A)*, **135**: 25-38.

Huang, X., Guo, F., Ke, C., and Wang, W. X., 2010. Responses of abalone *Haliotis diversicolor* to sublethal exposure of waterborne and dietary silver and cadmium. *Ecotoxicology and Environmental Safety*, **73**: 1130-1137.

Huang, X. X., Zhou, H. Q., and Zhang, H., 2006. The effect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the shrimp *Fenneropenaeus chinensis*. *Fish and Shellfish Immunology*, **20**: 750-757.

Jiang, W. D., Wu, P., Kuang, S. Y., Liu, Y., Jiang, J., Hu, K., Li, S. H., Tang, L., Feng, L., and Zhou, X. Q., 2011. *Myo*-inositol prevents copper-induced oxidative damage and changes in antioxidant capacity in various organs and the enterocytes of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquatic Toxicology*, **105**: 543-551.

Jorge, M. B., Loro, V. L., Bianchini, A., Wood, C. M., and Gillis, P. L., 2013. Mortality, bioaccumulation and physiological responses in juvenile freshwater mussels (*Lampsilis siliquoidea*) chronically exposed to copper. *Aquatic Toxicology*, **126**: 137-147.

Kaland, T., Andersen, T., and Hylland, K., 1992. Accumulation and subcellular distribution of metals in the marine gastropod *Nassarius reticulates* L. In: *Ecotoxicology of Metals in Invertebrates*. Dallinger, R., and Rainbow, P. S., eds., Lewis, London, 37-53.

Kappus, H., 1985. Lipid peroxidation: Mechanisms, analysis, enzymology and biological relevance. In: *Oxidative Stress*. Sies, H., ed., Academic Press, London, 273-310.

Langston, W. J., Bebianno, M. J., and Burt, G. R., 1998. Metal handling strategies in molluscs. In: *Metal Metabolism in Aquatic Environments*. Langston, W. J., and Bebianno, M. J., eds., Chapman and Hall, London, 219-283.

Li, L. J., Zhang, F., and Liu, X. M., 2005. Oxidative stress related enzymes in response to chromium (VI) toxicity in *Oxya chinensis* (Orthoptera: Acridoidae). *Journal of Environmental Science (China)*, **17**: 823-826.

Maria, V. L., and Bebianno, M. J., 2011. Antioxidant and lipid peroxidation responses in *Mytilus gallopro vincialis* exposed to mixtures of benzo (a) pyrene and copper. *Comparative Biochemistry and Physiology (C)*, **154**: 56-63.

Marr, J. C. A., Lipton, J., Cacela, D., Hansen, J. A., Bergman, H. L., Meyer, J. S., and Hogstrand, C., 1996. Relationship between copper exposure duration, tissue copper concentration, and rainbow trout growth. *Aquatic Toxicology*, **36**: 17-30.

Mazon, A. F., Nolan, D. T., Lock, R. A. C., Fernandes, M. N., and Wendelaar Bonga, S. E., 2004. A short-term *in vitro* gill culture system to study the effects of toxic (copper) and non-toxic (cortisol) stressors on the rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Toxicology in Vitro*, **18**: 691-701.

McGeer, J. C., Szebedinszky, C., McDonald, D. G., and Wood, C. M., 2000. Effects of sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout 1: Ionoregulatory disturbance and metabolic costs. *Aquatic Toxicology*, **50**: 231-243.

Meng, W., Qin, Y. W., Zheng, B. H., and Zhang, L., 2008. Heavy metal pollution in Tianjin Bohai Bay, China. *Journal of Environmental Sciences*, **20**: 814-819.

Mosleh, Y. Y., Paris-Palacios, S., and Biagianti-Risbourg, S., 2005. Metallothioneins induction and antioxidative response in aquatic worms *Tubifex tubifex* (Oligochaeta, tubificiadae) exposed to copper. *Chemosphere*, **64**: 121-128.

Ohkawa, H., Ohish, I. N., and Yagi, K., 1979. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, **95**: 351-363.

Palace, V. P., and Klaverkamp, J. F., 1993. Variation of hepatic enzymes in three species of freshwater fish from Precambrian shield lakes and the effect of cadmium exposure. *Comparative Biochemistry and Physiology (C)*, **104**: 147-154.

Reddy, P. S., 1997. Modulations in antioxidant enzymes in the gill and hepatopancreas of the edible crab *Scylla serrata* during exposure to cadmium and copper. *Fresenius Environmental Bulletin*, **6**: 589-597.

Reddy, P. S., and Bhagyalakshmi, A., 1994. Lipid peroxidation in the gill and hepatopancreas of *Oziotelphusa senex senex* fabricius during cadmium and copper exposure. *Bulletin of Environmental Contamination and Toxicology*, **53**: 704-710.

Reméo, D., Bennani, N., Gnassia-Barelli, M., Lafaurie, M., and Girard, J. P., 2000. Cadmium and copper display different responses towards oxidative stress in the kidney of the sea bass *Dicentrarchus labrax*. *Aquatic Toxicology*, **48**: 185-194.

Ruas, C. B. G., Carvalho, C. S., de Araujo, H. S. S., Espindola, E. L. G., and Fernandes, M. N., 2008. Oxidative stress biomarkers of exposure in the blood of cichlid species from a metal-contaminated river. *Ecotoxicology and Environmental Safety*, **71**: 86-93.

Şahin, M., Sağdıç, G., Elmas, O., Akpınar, D., Derin, N., Aslan, M., Agar, A., Alıcıgüzel, Y., and Yargıçoğlu, P., 2007. Effect of chronic restraint stress and alpha-lipoic acid on lipid peroxidation and antioxidant enzyme activities in rat peripheral organs. *Pharmacological Research*, **54**: 247-252.

Scalbert, A., Johnson, I. T., and Saltmarsh, M., 2005. Polyphenols: Antioxidants and beyond. *American Journal of Clinical Nutrition*, **81**: 215S-217S.

Sies, H., 1985. Oxidative stress: Introductory remarks. In: *Oxidative Stress*. Sies, H., ed., Academic Press, San Diego, 1-8.

Sies, H., 1999. Glutathione and its role in cellular functions. *Free Radical Biology and Medicine*, **27**: 916-921.

Sörensen, E. M., 1991. *Metal Poisoning in Fish*. CRC Press, Boston, 384pp.

Stohs, S. J., Bagchi, D., Hassoun, E., and Bagchi, M., 2000. Oxidative mechanisms in the toxicity of chromium and cadmium ions. *Journal of Environmental Pathology, Toxicology and Oncology*, **19**: 201-213.

Tamás, L., Valentovicová, K., Halusková, L., Huttová, J., and Mistrík, I., 2009. Effect of cadmium on the distribution of

hydroxyl radical, superoxide and hydrogen peroxide in barley root tip. *Protoplasma*, **236**: 67-72.

Thomas, P., and Juedes, M. J., 1992. Influence of lead on the glutathione status of Atalantic croaker tissues. *Aquatic Toxicology*, **23**: 11-30.

Thomas, P., and Wofford, H. W., 1984. Effects of metals and organic compounds on hepatic glutathione, cysteine, and acid soluble thiol levels in mullet (*Mugil cephalus* L.). *Toxicology and Applied Pharmacology*, **76**: 172-182.

Thomas, P., and Wofford, H. W., 1993. Effects of cadmium and Aroclor 1254 on lipid peroxidation, glutathione peroxidase activity, and selected antioxidants in Atlantic croaker tissues. *Aquatic Toxicology*, **27**: 159-178.

Thomas, P. T., Wofford, H. W., and Neff, J. M., 1982. Effect of cadmium on glutathione content of mullet (*Mugil cephalus*) tissues. In: *Physiological Mechanisms of Marine Pollutant Toxicity*. Vernberg, W. B., *et al*., eds., Academic Press, New York, 109-125.

Tort, L., Kargacin, B., Torres, P., Giralt, M., and Hidalgo, J., 1996. The effect of cadmium exposure and stress on plasm cortisol, metallothionein levels and oxidative status in rainbow trout (*Oncorhynchus mykiss*) liver. *Comparative Biochemistry and Physiology (C)*, **114**: 29-34.

Tsai, J. W., Chou, Y. H., Chen, B. C., Liang, H. M., and Liao, C. M., 2004. Growth toxicity bioassays of abalone *Haliotis diversicolor supertexta* exposed to waterborne zinc. *Bulletin of Environmental Contamination and Toxicology*, **72**: 70-77.

Upadhyay, R., and Panda, S. K., 2010. Zinc reduces copper toxicity induced oxidative stress by promoting antioxidant defense in freshly grown aquatic duckweed *Spirodela polyrhiza* L. *Journal of Hazardous Materials*, **175**: 1081-1084.

Verlecar, X. N., Jena, K. B., and Chainy, G. B. N., 2008. Modulation of antioxidant defences in digestive gland of *Perna viridis* (L.), on mercury exposures. *Chemosphere*, **71**: 1977-1985.

Viarengo, A., Canesi, L., Pertica, M., Poli, G., Moore, M. N., and Orunesu, M., 1990. Heavy metal effect on lipid peroxidation in the tissues of *Mytilus galloprovincialis. Comparative Biochemistry and Physiology (C)*, **97**: 37-42.

Vieira, L. R., Gravato, C., Soares, A. M. V. M., Morgado, F., and Guilhermino, L., 2009. Acute effects of copper and mercury on the estuarine fish *Pomatoschistus microps*: Linking biomarkers to behavior. *Chemosphere*, **76**: 1416-1427.

Vincent, R., Boudreau, J., Nadeau, D., Fournier, M., Krrzystyniak, K., Trottier, B., and Chevalier, G., 1989. Lipid peroxidation in rat lungs following an acute inhalation exposure to cadmium chloride. *Journal Aerosol Medicine*, **2**: 349- 356.

Wang, G. D., Liu, B. Z., Tang, B. J., Zhang, T., and Xiang, J. H., 2006. Pharmacological and immunocytochemical investigation of the role of catecholamineson larval metamorphosis by beta-adrenergic-like receptor in the bivalve *Meretrix meretrix*. *Aquaculture*, **258**: 611-618.

Wang, N., Ingersoll, C. G., Greer, I. E., Hardesty, D. K., Ivey, C. D., Kunz, J. L., Brumbaugh, W. G., Dwyer, F. J., Roberts, A. D., Augspurger, T., Kane, C. M., Neves, R. J., and Barnhart, M. C., 2007a. Chronic toxicity testing of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry*, **26**: 2048-2056.

Wang, N., Ingersoll, C. G., Hardesty, D. K., Ivey, C. D., Kunz, J. L., May, T. W., Dwyer, F. J., Roberts, A. D., Augspurger, T., Kane, C. M., Neves, R. J., and Barnhart, M. C., 2007b. Acute toxicity f copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry*, **26**: 2036-2047.

Warner, H. R., 1994. Superoxide dismutase, aging and degenerative disease. *Free Radical Biology and Medicine*, **17**: 249-258.

Winterbourn, C. C., 1982. Superoxide-dependent production of hydroxyl radicals in the presence of iron salts (Letter). *Biochemical Journal*, **205**: 463.

Xu, H. Z., Zhou, C. G., Ma, Y. A., Shang, L. S., Yao, Z. W., and Li, H., 2000. Environmental quality of deposits in offshore zone of China. *Environmental Protection in Transportation*, **21**: 16-18.

Xu, X. D., Lin, Z. H., and Li, S. Q., 2005. The studied of the heavy metal pollution of Jiaozhou Bay. *Marine Sciences*, **29**: 48-53.

Yu, B. P., 1994. Cellular defenses against damage from reactive oxygen species. *Physiological Reviews*, **74**: 139-162.

Yu, R. L., Yuan, X., Zhao, Y. H., Hu, G. R., and Tu, X. L., 2008. Heavy metal pollution in intertidal sediments from Quanzhou Bay, China. *Journal of Environmental Science*, **20**: 664-669.

Yuan, C. G., Shi, J. B., He, B., Liu, J. F., Liang, L. N., and Jiang, G. B., 2004. Speciation of heavy metals in marine sediments from the East China Sea by ICP-MS with sequential extraction. *Environment International*, **30**: 769-783.

(Edited by Qiu Yantao)