Chinese Journal of Oceanology and Limnology
Vol. 28 No. 4, P. 849-855, 2010
DOI: 10.1007/s00343-010-9127-x

The influence of cadmium on the antioxidant enzyme activities in polychaete *Perinereis aibuhitensis* Grube (Annelida: Polychaeta)*

YUAN Xiutang (袁秀堂)[†,††], CHEN Aihua (陈爱华)[†], ZHOU Yibing (周一兵)[†,**], LIU Haiying (刘海映)[†], YANG Dazuo (杨大佐)[†]

[†] *Key Laboratory of Marine Bio resourse Sustainable Utilization in Liaoning Province' University, Dalian Ocean University, Dalian 116023, China*

[††] *National Marine Environmental Monitoring Center, Dalian 116023, China*

Received Jul. 28, 2009; revision accepted Dec. 22, 2009

Abstract The infaunal polychaete *Perinereis aibuhitensis* Grube, distributed widely along Asian coasts and estuaries, is considered a useful animal model in ecotoxicological tests and a promising candidate in biomonitoring programs. This paper deals with the activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidases (GSH-Px) in infaunal polychaete *P. aibuhitensis* exposed to a series of sublethal water-bound cadmium (Cd) concentrations (0, 0.34, 1.72, 3.44, 6.89, and 17.22 mg L^{-1}) under a short-term exposure (1–8 d). The results indicate that the SOD and GSH-Px activities in *P. aibuhitensis* are stimulated first and then renewed to the original level. The CAT activity of worms decreases at an earlier exposure time but increases to the control values at a later exposure time. Our study suggests that Cd can interfere with the antioxidant defense system of *P. aibuhitensis*. However, the changes in antioxidant enzyme activities for this species do not show the best promise as biomarkers in Cd biomonitoring of estuarine and coastal zones because weak or non-dose-effect relationships between the antioxidant enzymes activities and Cd levels are found.

Keyword: Polychaete (*Perinereis aibuhitensis*); cadmium; antioxidant enzyme; biomarker; biomonitoring.

1 INTRODUCTION

Estuaries and coastal zones are some of the most productive and economically important ecosystems, but they are always contaminated by heavy metals derived from anthropogenic activities due to the fast development around these economic regions (Kennish, 2002; Singh et al., 2007). Heavy metals such as mercury (Hg), cadmium (Cd), copper (Cu), arsenic (As), and zinc (Zn) are either discharged directly into these unique environments or are delivered by rivers and streams. It is well known that several environmental contaminants including heavy metals can induce oxidative stress in marine animals by generating reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\cdot$), and superoxide anion ($O_2^{\cdot-}$) during their cellular metabolism (Newman et al., 2003; Bocchetti et al., 2004; Ferreira-Cravo et al., 2007). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidases (GSH-Px) play an important role in defense systems against ROS-mediated toxicity (Newman et al., 2003; Ferreira-Cravo et al., 2007). Furthermore, for biomonitoring purpose, antioxidant enzymes have been regarded as potential exposure biomarkers for metal pollution (Lam et al., 2003).

Polychaetes are considered as ecologically significant species in estuarine and coastal environments mainly because (1) they can scavenge detritus and organic matters on the sediment surface and play a key role in nutrient cycling in water-sediment coupling (Davey et al., 1995; Durou et al., 2005, 2007), and (2) they function as an important food source for many benthic fishes and migratory birds, therefore contributing to the

* Supported by the High Technology Research and Development Program of China (863 Program. No. 2006AA10Z410), the National Marine Public Welfare Research Project (No. 200805069), and the National Natural Science Foundation of China (Nos. 30571419, 30901107)
** Corresponding author: ybzhou@dlfu.edu.cn

bioampification of metals through the trophic chains (Scaps, 2002; Sun et al., 2006). Several polychaetes have been used as biomonitors for toxicological studies because they are deposit-feeders, have sensitivity and resistance to contaminants, and are relatively easy to collect, handle, culture, and transport (Scaps, 2002; King et al., 2004; Casado-Martínez et al., 2008). Moreover, polychaetes have a relatively short generation time that makes them ideal organisms for studying the effects of toxicants on differently developed stages (Mauri et al., 2003; Lau et al., 2007; Gopalakrishnan et al., 2007; 2008).

The deposit feeder *Perinereis aibuhitensis* (Grube 1878) is widely abundant along Asian coasts and estuaries including China, India, Indonesia, and the Philippines due to its eurythermal and euryhaline characteristics (Wu et al., 1981; Yang et al., 1988). It prefers to inhabit in mud and sandy beaches and is always the dominant species in intertidal zones. Many studies have shown that this species can easily be cultivated and bred in a laboratory (Wang et al., 2004, 2005; Zhou et al., 2007). These facts point out that *P. aibuhitensis* is a good candidate for studying its ecotoxicology through bioassays in the laboratory. Even with this background in mind, however, there has been little information available on the ecotoxicology in polychaete *P. aibuhitensis* until now (e.g., Wang et al., 2007, 2008). This scarce information on the ecotoxicology in this species seriously retards the biomonitoring technology development and usefulness of estuarine and coastal zone pollution monitoring.

The aim of this work is to evaluate under laboratory conditions the antioxidant responses of the polychaete *P. aibuhitensis* to Cd. Moreover, such indices are analyzed to determine whether they can become useful biomarkers in the environmental biomonitoring of estuarine and coastal zones or not.

2 MATERIALS AND METHODS

2.1 Animal collection and adaptation

The polychaete *P. aibuhitensis* was collected at a low tide from beach of Zhuanghe coast (a relatively unpolluted site) on May 2005. The environmental temperature was around 14.8°C at the time of animal collection. The animals were transferred in an ice cooler, along with the sediment from the same sampling site, from the field to the laboratory (this process lasted for 4 to 5 h) where they were transferred to glass tanks (50 cm×40 cm×40 cm) with sediment and seawater. After one week of adaptation, the water temperature was gradually adjusted at a rate of about 1°C d^{-1}, then the animals were kept at the test regime (20±0.5°C) for one week of acclimatization before being placed under experimental conditions. During the acclimatization, aeration was provided continuously, with the animal density set at ca. 400 ind. m^{-2}. The sea water was changed completely daily, and worms were fed excessively with a commercial formulated diet (13.0% moisture, 44.7% crude protein, 26.1% crude lipid, and 10.8% ash) for culturing flounder.

2.2 Preparation of sand

Sea sand (¢: 60–2 000 μm) was collected in the seashore in the worm sampling site of origin and was used in the experiment to provide refuge for *P. aibuhitensis*. The sand was rinsed with sea water, dried at 65°C, and then combusted in a muffle furnace at 550°C for 12 h to get rid of organic matter. After these processes, the sand was acidified with concentrated HNO_3 (10% HNO_3 [V/V]) and then rinsed and dried at 65°C again for further use.

2.3 Cd exposure

One hundred eighty worms (0.75±0.18 g) were selected from the acclimated animals and were averagely placed into sixty 2 L plastic beakers containing 1 000 ml aerated sea water and a 2–3 cm layer of sand (ca. 1 000 g). All the containers including the beakers used in the experiments had been earlier soaked in 10% HNO_3 [V/V] for 24 h to eliminate any adsorption of metals. The tested Cd was used as $CdCl_2$. 2.5 H_2O (analytical grade). A 1 g L^{-1} Cd solution was prepared as storage solution and then it was diluted for various concentrations in the experiments. The solvent carrier was sea water filtered by a composite sand filter from Heishijiao seashore in Dalian City, Liaoning Province in China. The background levels of heavy metals in the carrier were Cu^{2+} 0.38 μg L^{-1}, Zn^{2+} 75 μg L^{-1}, Pb^{2+} 0.27 μg L^{-1}, and Cd^{2+} 0.06 μg L^{-1}. Exposed Cd concentrations were chosen as 0 mg L^{-1}(control, C), 0.34 mg L^{-1}(level 1, L_1), 1.72 mg L^{-1}(level 2, L_2), 3.44 mg L^{-1}(level 3, L_3), 6.89 mg L^{-1}(level 4, L_4), and 17.22 mg L^{-1}(level 5, L_5), which were set by multiplying 34.44 mg L^{-1} (48h$LC_{50,}$ which was measured in our preliminary experiment) by 0, 0.01, 0.05, 0.1, 0.2, and 0.5 (Zhou et al., 1989). Cd-contaminated sea water was renewed daily to replenish the metal and avoid the accumulation of excretory products. The control and Cd-exposed worms (10 replicates in which 3 worms were tested

for each treatment) were sampled at days 1, 2, 4, 6, and 8 for antioxidant enzymes analysis. During the experiments, the water salinity was 31–32 ppt, the dissolved oxygen was above 6.5 mg L^{-1}, and the pH was at 8.25±0.10. Natural photoperiod in the laboratory (ca. 14L: 10D) was used. The beakers were checked daily and dead worms were eliminated from the beakers as soon as possible.

2.4 Preparation of tissue extract and assay of enzyme activity

During each sampling time on days 1, 2, 4, 6, and 8, three worms exposed to each concentration of the tested contaminant were randomly selected from the beakers to determine the enzyme activities. First, the worms were rinsed with distilled water, wiped using filter paper, and weighed as wet weight (ww). Second, the three selected worms in each treatment were homogenized individually in 9 volumes (the ratio of weight to volume) of 50 mmol/L ice-cold Na-phosphate buffer solution, pH 7.8, using a manual glass homogenizer maintained in an ice bath. The homogenates were then centrifuged at 4 200 *g* for 10 min at 4°C. The supernatants were collected for further determination of enzyme activities.

The activities of SOD, CAT, and GSH-Px were determined using commercial detection kits (Nanjing Jiancheng Bioengineering Institute, China). SOD activity was determined by hydroxylamine assay developed from xanthine oxidase assay. One SOD unit was defined as the amount of enzyme needed to inhibit 50% of cytochrome *c* reduction per min and per mg of proteins at 37°C. CAT activity was measured by the ammonium molybdate method, and one CAT unit represented the amount of enzyme needed to degrade 1 μmol of H_2O_2 per min and per mg of proteins at 37°C. Finally, GSH-Px was determined by spectrophotometry to test the yellow 5-sulf-bi-nitro-benzoic acid (the product of GSH and DTNB accelerated by GSH-Px) at 412 nm. One GSH-Px unit was defined as the amount of enzyme necessary to oxidize 1 μmol of GSSG per min and per mg of protein at 37°C. The protein content of homogenates in the test worms was measured by the dye-binding method according to Bradford (1976) using bovine serum albumin as the standard and expressed as mg/g ww (wet weight). All enzyme activity data were related to the protein content of homogenates in the test worms, and the activity of the enzymes was expressed as U/mg protein.

2.5 Statistic analysis

One-way ANOVA was performed using the SPSS version 11.0 statistical package for Microsoft Windows to determine the differences in SOD, CAT, and GSH-Px among the Cd concentrations during the same sampling day. Duncan's multiple range tests were used to test the differences in the treatments. Partial correlation analysis was used to test the co-relationships between SOD, CAT, GSH-Px, and the exposed Cd concentrations. The differences were considered significant at a probability level of $P<0.05$, respectively.

3 RESULTS

3.1 SOD activity

The SOD activities in the test worms under different doses but the lowest dose (L_1) of Cd exposure were higher than those in the control worms (*$P<0.05$) at days 1 and 2 (Fig.1). Significant differences in the SOD activities of the test worms were observed in L_2, L_3, L_4, and L_5 treatments at days 1 and 2 (*$P<0.05$); however, there were no obvious dose-effect relationships between the SOD activities and Cd levels (r=0.0243, $P>0.05$). No significant differences were found in the SOD activities of the test worms in all treatments (including control treatment), although the enzyme activities of some treatments increased slightly in comparison with the control at days 4, 6, and 8 ($P>0.05$).

3.2 CAT activity

The CAT activities in the polychaete *P. aibuhitensis* under different doses of Cd exposure are presented in Fig.2. The CAT activities in the test worms were significantly lower than those in the control worms (*$P<0.05$) at days 1, 2, and 4 with the exception of the worms under the lowest dose of Cd exposure (L_1, 0.34 mg.L^{-1}) at days 1 and 4 ($P>0.05$). There were no significant differences in CAT activity in L_2, L_3, L_4, and L_5 treatments at days 1, 2, and 4 ($P>0.05$). Additionally, a non-significant decrease ($P>0.05$) in CAT activity of the test worms was found with the increased dose of Cd exposure (besides L_5 Cd exposure) at days 6 and 8. Partial correlation analysis showed that the CAT activities of the test animals were weakly correlated with their waterborne Cd levels (r=-0.2498, $P<0.05$).

3.3 GSH-Px activity

The GSH-Px activities in the test worms (Fig.3) showed the same tendency as the SOD activities. The GSH-Px activities in the test worms under different doses of Cd exposure were higher than those in the control (*$P<0.05$), with the exception of those in the

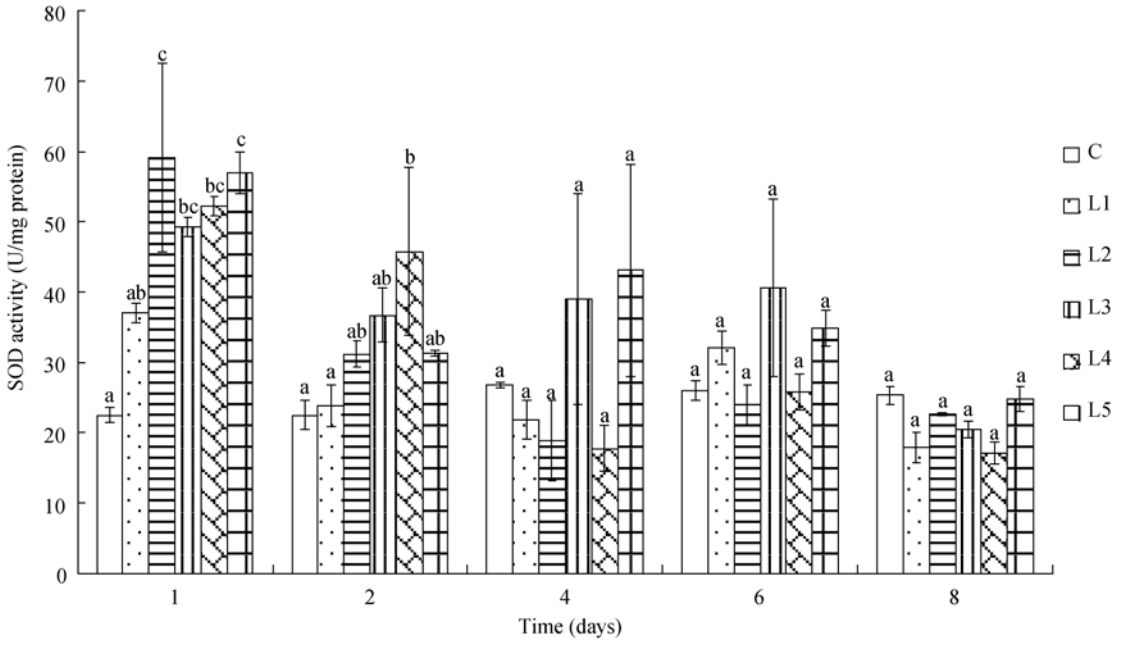

Fig.1 Change in SOD activity of *P. aibuhitensis* under different levels of Cd exposure

Values (mean±S.E., n=3) with different letters in the same cluster indicate significant differences (*P<0.05)

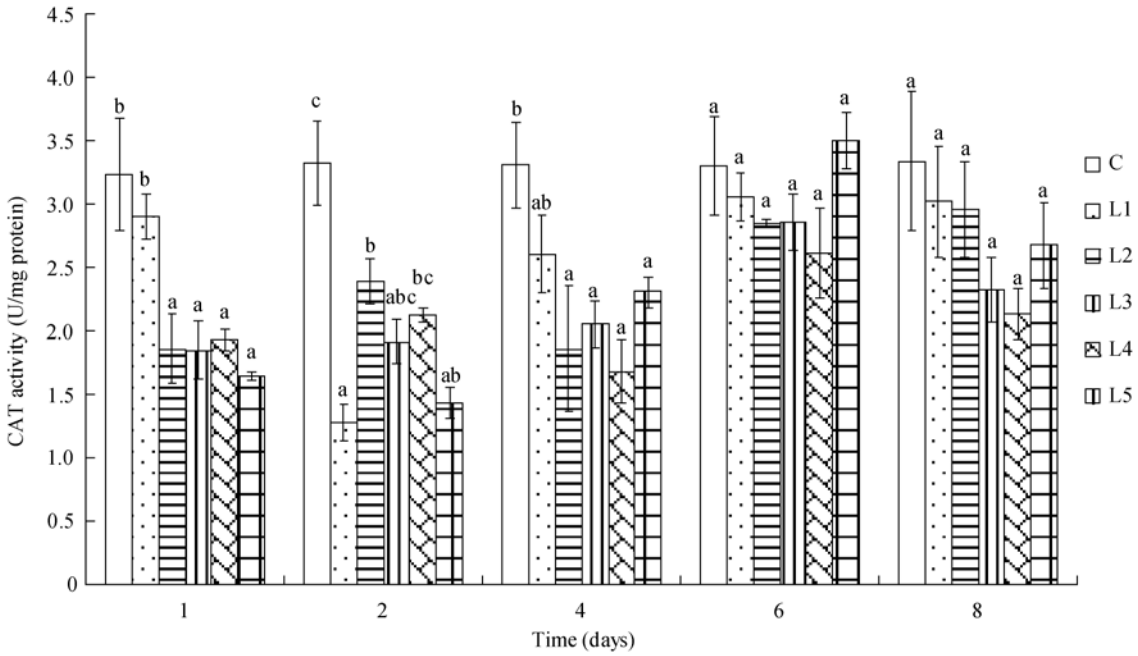

Fig.2 Change in CAT activity of *P. aibuhitensis* under different levels of Cd exposure

Values (mean±S.E., n=3) with different letters in the same cluster indicate significant differences (*P<0.05)

L_2 treatment (P>0.05) at day 1. Significant differences in the GSH-Px activities of the test worms were detected in L_1, L_2, L_3, L_4, and L_5 treatments at day 1 (*P<0.05). At day 2, the higher GSH-Px activities in the L_3 treatment were only observed (*P<0.05); however, there were no significant differences in the other treatments (control, L_1, L_2, L_4, and L_5) (P>0.05). Moreover, no significant differences were found in the GSH-Px activities of the test worms in all treatments (including the control treatment) at days 4 and 8 (P>0.05). Additionally, the highest value (0.482 9 U/mg protein) and lowest value (0.318 7 U/mg protein) of GSH-Px activity (*P<0.05) were found in L_2 and L_4 treatments, respectively, at day 8, but there was no significant difference in the other treatments (control, L_1, L_3, and L_5) (P>0.05). Dose-effect relationships between the GSH-Px activities and Cd levels were not found (r=0.046 1, P>0.05).

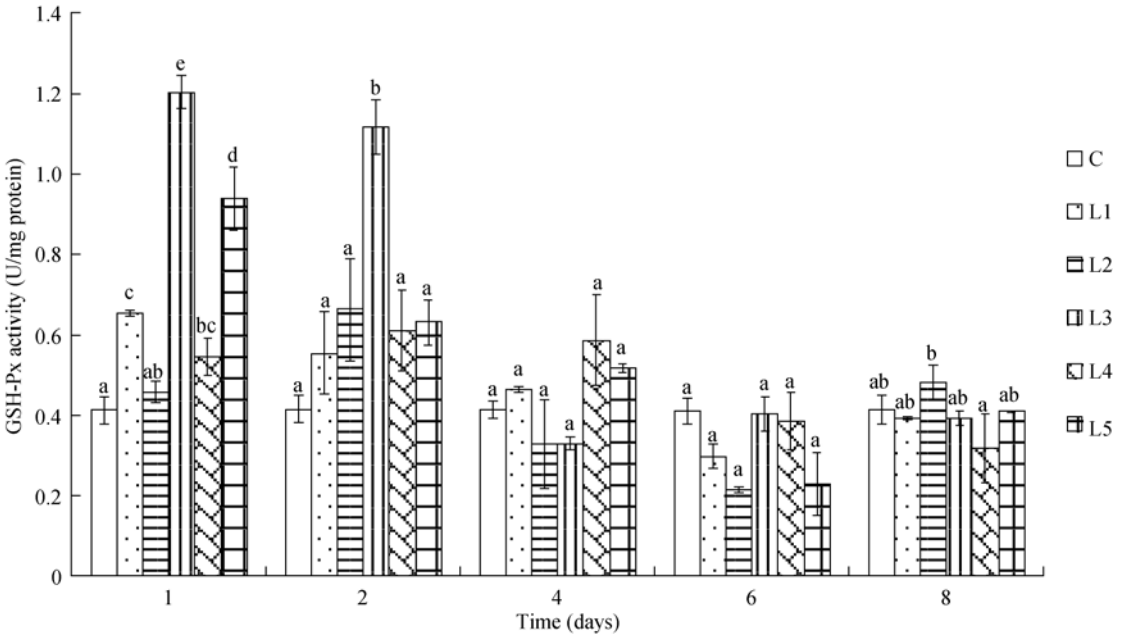

Fig.3 Change in GSH-Px activity of *P. aibuhitensis* under different levels of Cd exposure

Values (mean±S.E., n=3) with different letters in the same cluster indicate significant differences (*P<0.05)

4 DISCUSSION

Cd pollution in estuarine or coastal environments is a global concern because it is biologically a non-essential element and has severe toxic effects on marine animals even at a low concentration (Singh et al., 2007; Sun et al., 2008). Contrary to redox active metals such as Cu and iron, Cd cannot directly induce ROS generation through Harber-Weiss and Fenton reaction (Stohs et al., 1995; Sandrini et al., 2008). However, many authors have observed an increase in the oxidative damage to macromolecules in aquatic animals after their exposure to Cd (Wang et al., 2004; Sun et al., 2008; Sandrini et al., 2008). Two different mechanisms could be related to the increment in ROS generation under Cd stress: (1) Cd can inhibit the electron transfer chain in the mitochondria, which may increase its ROS generation (Wang et al., 2004), and (2) alternatively, the generation of ROS after Cd exposure is attributed to the interference of Cd in the cellular antioxidant defense system (Stohs et al., 1995; Waisberg et al., 2003). Our study confirms that Cd can interfere with the antioxidant defense system of the polychaete *P. aibuhitensis*, which can be an indicator of ROS generation in *P. aibuhitensis*.

The antioxidant enzymes play an important role in protecting cellular systems from oxidative damages induced by xenobiotics through a neutralizing ROS. The current study demonstrated that a short-term (8 days) exposure to sublethal waterborne Cd concentrations causes activity alterations of the antioxidant enzymes in *P. aibuhitensis*. SOD is one of the most important enzymes that protect organisms from oxidative damages by transforming the superoxide anion ($O_2^{\cdot -}$) into hydrogen peroxide (H_2O_2). The enhanced activities of SOD in many polychaetes were reported such as in *N. diversicolor* (Sun et al., 2008; Moreira et al., 2006) and *L. acuta* (Geracitano et al., 2004a, b) when the test worms were exposed to heavy metals and/or petroleum hydrocarbons (PHCs). The present study also observed that the SOD activities in the test worms under different concentrations (1.72–17.22 mg L^{-1}) of Cd exposure were significantly higher than those in the worms of the control at days 1 and 2 (Fig.1), which is similar to previous studies on *N. diversicolor* (Moreira et al., 2006; Sun et al., 2008). However, during the period of days 4 to 8, this increase in SOD activity thereafter decreased to the control value. The consequent decrease in SOD activity may be a result of the elimination of O_2^- by antioxidant enzymes.

CAT can protect organisms from oxidative damages by making more disproportionate H_2O_2 into H_2O, a response that can be considered an adaptation of polychaete to overcome stress due to contaminants and to prevent their toxicity (Geracitano et al., 2004a, b). In the present study, a trend was found in that the CAT activities of polychaete *P. aibuhitensis* decreased at days 1 to 4 under different levels of Cd (0.34–17.22 mg L^{-1}) exposure compared with those of the control worms, but these reverted to the control

values from days 6 to 8 of exposure (Fig.2). The results agree will with those previously reported. For example, the CAT activities of polychaete *Laeonereis acuta* decreased after 8 h exposure to Cd (5 and 100 μg L^{-1}) (Sandrini et al., 2006) but were not affected by exposure to the same Cd concentrations at day 7's longer period of test (Sandrini et al., 2008). However, the significant increase in CAT activity in the same species of *L. acuta* in both field samples (Geracitano et al., 2004b) and acute (4d, 125 and 250 μg L^{-1}) or chronic (14d, 62.5 μg L^{-1}) exposure to Cu in the laboratory (Geracitano et al., 2004a) was also reported. Thus, CAT activities under the stress of contaminants have shown different responses in polychaetes. These variable and transient CAT responses in polychaetes may be mainly attributed to the different contaminants and the long or short stress times.

Little information is available on the GSH-Px activity in polychaetes after exposure to heavy metals. Similar to the SOD activity, the GSH-Px activity in *P. aibuhitensis* was detected to have increased values in the earlier days of the Cd stress experiment (days 1 to 2), but this consequently reverted to the control level at days 4 to 8. GSH-Px is considered a key enzyme in cells that can decompose peroxide and protect the integrity of the structure and function of the membrane by cleaning up the harmful metabolite of peroxide and breaking off the chain reaction of lipid peroxidation. With the deoxidizing process in cells continued, a decrease in GSH-Px activity in the test polychaete could be inevitable.

Although the antioxidant activities have been proposed as biochemical biomarkers to assess the toxic effects of contaminants in marine polychaetes in both laboratory (Geracitano et al., 2004a; Sun et al., 2008; Sandrini et al., 2008; Zhang et al., 2008) and field studies (Geracitano et al., 2004b; Bocchetti et al., 2004; Ferreira-Cravo et al., 2007), a debate on the usefulness of antioxidant responses to indicate contaminant-mediated stress in polychaete was found. For example, Sun et al. (2008) found that the antioxidant responses in *N. diversicolor* had significant correlations with heavy metal and/or petroleum hydrocarbons. Additionally, Geractiano et al. (2004a, b) in both field and laboratory studies suggested that the polychaete *L. acuta* from a polluted site showed higher antioxidant responses than those from an unpolluted site, and worms from a polluted site were more susceptible to oxidative stress conditions. However, the studies performed by Sandrini et al. (2006, 2008) found that the antioxidant enzyme responses in *L. acuta* did not show significant positive correlations between antioxidant enzyme activities and pollutant doses. Our results showed that as a kind of endogenous ROS eliminator, the antioxidant enzymes SOD, CAT, and GSH-Px could counteract excessive ROS to sustain super peroxide balance and protect membrane stability under the stress of a contaminated environment. However, this detoxification is somewhat limited, and the antioxidant enzymes responses seem to be short-term stress effects, as we noticed only a transient increase or decrease in antioxidant enzyme activity between days 1 to 4, but this reverted at days 4 to 8. More importantly, a weak or non-dose-effect relationship was found between antioxidant enzymes activities and Cd concentrations in this study. Thus, the responses of antioxidant enzymes CAT, SOD, and GSH-Px in *P. aibuhitensis* could reflect the Cd contaminant concentrations in the environment to some extent, but they did not demonstrate to be promising biomarkers in coastal environment monitoring.

References

Bocchetti R, Fattorini D, Gambi M C, Regoli F. 2004. Trace metal concentrations and susceptibility to oxidative stress in the polychaete *Sabella spallanzanii* (Gmelin) (Sabellidae): potential role of antioxidants in revealing stressful environmental conditions in the Mediterranean. *Arch. Environ. Contam. Toxicol.*, **46**: 353-361.

Bradford M M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**(7): 248-254.

Casado-Martínez M C, Branco V, Vale C, Ferreira A M, DelValls T A. 2008. Is *Arenicola marina* a suitable test organism to evaluate the bioaccumulation potential of Hg, PAHs and PCBs from dredged sediments? *Chemosphere*, **70**: 1 756-1 765.

Davey J T, Waston P J. 1995. The activity of *Nereis diversicolor* (polychaeta) and its impact on the nutrient fluxes in estuarine waters. *Ophelia*, **41**: 55-70.

Durou C, Smith B D, Roméo M, Rainbow P S, Mouneyrac C, Mouloud M, Gnassia-Barelli M, Gillet P, Deutch B, Amiard-Triquet C. 2007. From biomarkers to population responses in *Nereis diversicolor:* Assessment of stress in estuarine ecosystems. *Ecotoxicol. Environ. Saf.*, **66**(3): 402-411.

Durou C, Mouneyrac C, Amiard-Triquet C. 2005. Tolerance to metals and assessment of energy reserves in the polychaete *Nereis diversicolor* in clean and contaminated estuarine. *Environ. Toxicol.*, **20**: 23-31.

Ferreira-Cravo M, Piedras F R, Moraes T B, Ferreira J L R, de Freitas D P S, Machado M D, Geracitano L A, Monserrat J M. 2007. Antioxidant responses and reactive oxygen species generation in different body regions of the estuarine polychaeta *Laeonereis acuta* (Nereididae).

Chemosphere, **66**: 1 367-1 374.

Geracitano L A, Bocchetti R, Monserrat J M, Rogoli F, Bianchini A. 2004a. Oxidative stress responses in two populations of *Laeonereis acuta* (Polychaeta, Nereididae) after acute and chronic exposure to copper. *Mar*. *Environ*. *Res*., **58**: 1-17.

Geracitano L A, Monserrat J M, Bianchini A. 2004b. Oxidative stress in *Laeonereis acuta* (Polychaeta, Nereididae): environmental and seasonal effects. *Mar*. *Environ*. *Res*., **58**: 625-630.

Gopalakrishnan S, Thilagam H, Raja P V. 2007. Toxicity of heavy metals on embryogenesis and larvae of the marine sedentary polychaete *Hydroides elegans*. *Arch*. *Environ*. *Contam*. *Toxicol*., **52**: 171-178.

Gopalakrishnan S, Thilagam H, Raja P V. 2008. Comparison of heavy metal toxicity in life stages (spermiotoxicity, egg toxicity, embryotoxicity and larval toxicity) of *Hydroides elegans*. *Chemosphere*, **71**: 515-528.

Kennish M J. 2002. Environmental threats and environmental future of estuaries. *Environ*. *Conserv*., **29**: 78-107.

King C K, Dowse M C, Simpson S L, Jolley D F. 2004. An assessment of five Austrilian polychaetes and bivalves for use in whole-sediment toxicity tests: toxicity and accumulation of copper and zinc from water and sediment. Archives of *Environ*. *Contam*. *Toxicol*., **47**: 314-323.

Lam K S, Gray J S. 2003. The use of biomarkers in environmental monitoring programmes. *Mar*. *Pollut*. *Bull*., **46**: 182-186.

Lau M C, Chan K M, Leung K M Y, Luan T G, Yang M S, Qiu J W. 2007. Acute and chronic toxicities of tributyltin to various life stages of the marine polychaete *Hydroides elegans*. *Chemosphere*, **69**: 135-144.

Mauri M, Baraldi E, Simonini R. 2003. Effects of zinc exposure on the polychaete *Dinophilus gyrociliatus*: a life-table response experiment. *Aquat*. *Toxicol*., **65**: 93-100.

Moreira S M, Lima I, Ribeiro R, Guilhermino L. 2006. Effects of estuarine sediment contamination on feeding and on key physiological functions of the polychaete *Hediste diversicolor*. Laboratory and in situ assays. *Aquat*. *Toxicol*., **78**: 186-201.

Newman M C, Unger M A. 2003. Fundamentals of Ecotoxicology. Second Edition. Lewis Publisher, CRC Press LLC, 319pp.

Sandrini J Z, Lima J V, Regoli F, Fattorini D, Notti A, Marins L F, Monserrat J M. 2008. Antioxidant responses in the nereidid *Laeonereis acuta* (Annelida, Polychaeta) after cadmium exposure. *Ecotoxicol*. *Environ*. *Saf*., **70**(1): 115-120.

Sandrini J Z, Regoli F, Fattorini D, Notti A, Ferreira Inácio A, Linde-Arias A R, Laurino J, Bainy A C D, Marins L F, Monserrat J M. 2006. Temporal responses to cadmium in the estuarine polychaete *Laeonereis acuta* (Polychaeta, Nereididae): subcellular distribution and oxidative stress generation. *Environ*. *Toxicol*. *Chem*., **25**(5): 1 337-1 344.

Scaps P. 2002. A review of the biology, ecology and potential use of the ragworm *Hediste diversicolor* (O. F. Müller) (Nereidida, Polychaeta). *Hydrobiologia*, **470**: 203-218.

Singh R K, Chavan S L, Sapkale P H. 2007. Heavy metal concentrations in water, sediments and body tissues of red worm (*Tubifex* spp.) collected from natural habitats in Munbai, India. *Environ*. *Monit*. *Assess*., **129**: 471-481.

Stohs S J, Bagchi D. 1995. Oxidative mechanisms in the toxicology of metal irons. *Free Radical Biol*. *Med*., **18**: 321-336.

Sun F, Zhou Q. 2006. Research advance in characteristics and mechanisms of *nereis diversicolor* endurance against environmental pollution, *Chinese J*. *Appl*. *Ecol*., **17**(3): 530-534. (in Chinese with English abstract)

Sun F, Zhou Q. 2008. Oxidative stress biomarkers of the polychaete *Nereis diversicolor* exposed to cadmium and petroleum hydrocarbons. *Ecotoxicol*. *Environ*. *Saf*., **70**(1): 106-114.

Waisberg M, Joseph, Hale B, Bayersmann D. 2003. Molecular and cellular mechanisms of cadmium carcinogenesis. *Toxicology*, **192**: 95-117.

Wang J, Zhou Q, Zhang Q, Zhang Y. 2007. Toxic effects of petroleum hydrocarbons, copper and cadmium on polychaete *Perinereis aibuhitensis* Grube and on its responses in acetycholinesterase activity. *Environ*. *Sci*., **28**(8): 1 796-1 801.(in Chinese with English abstract)

Wang J, Zhou Q, Zhang Q, Zhang Y. 2008. Single and joint effects of petroleum hydrocarbons and cadmium on the polychaete *Perinereis aibuhitensis* Grube. *J*. *Environ*. *Sci*., **20**(1): 68-74.

Wang L, Chen A H, Zhao X, Wang L, Zhang J C, Zhou Y B. 2004. Effects of the temperature and body weight on respiration and excretion in *Perinereis aibuhitensis* Grube. *J*. *Dalian Fish*. *Univ*., **19**(3): 176-181. (in Chinese with English abstract)

Wang L, Chen A H, Zhao X, Wang L, Zhang J C, Zhou Y B. 2005. Preliminary studies on the diurnal variation of metabolism of *Perinereis aibuhitensis* Grube. *J*. *Fish*. *China*, **29**(1): 48-54(in Chinese with English abstract).

Wang Y, Fang J, Leonard S S, Rao K M K. 2004. Cadmium inhabits the electron transfer chain and induces reactive oxygen species. *Free Radical Biol*. *Med*., **36**: 1 434-1 4443.

Wu B, Sun R, Yang D. 1981. The Neieidae (Polychaetous Annelids) of the Chinese coast, Marine Publishing House, Beijing. p.171-174. (in Chinese)

Yang D, Sun R. 1988. Polychaetous annelids commonly seen from the Chinese waters Agriculture publishing house, Beijing. p.45-46. (in Chinese)

Zhang N, Zhou Q X, Li T, Luo Y. 2008. Ecotoxic effects of oxidation hair dyes on *Perinereis aibuhitensis* Grube and its enzyme activities. *Asian J*. *Ecotoxicol*., **3**(1), 65-71. (in Chinese with English abstract)

Zhou Y X, Zhang Z S. 1989. Toxicity test methods of aquatic organisms. Agriculture Publishing House, Beijing, 266pp. (in Chinese)

Zhou Y B, Yang D Z, Guang Z C, Xu X Z, Zhao F F. 2007. Technique for artificial breeding and culture of *Perinereis aibuhitensis* in ponds. *Fish*. *Sci*., **26**(3): 150-153. (in Chinese with English abstract)