

## Cadmium Bioaccumulation and its Effect on Antioxidant Defense System of Japanese Scallop *Mizuhopecten yessoensis*

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**Abstract:** This research aimed to investigate the accumulation of Cadmium (Cd) and the antioxidant defense system (Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx)) in gill and digestive gland of *Mizuhopecten yessoensis* exposed to Cd (final Cd doses of 0, 0.05, 0.1 and 0.2 mg L<sup>-1</sup>) for 14 days. The uptake of Cd in both tissues via seawater displayed a simple linear relationship with the exposure of Cd concentration and a curvilinear change with time of extending. The maximal accumulation was observed at day 10 in gill in the 0.2 mg L<sup>-1</sup> group (134.20 µg g<sup>-1</sup>) and day 14 in digestive gland in the 0.1 mg L<sup>-1</sup> group (109.20 µg g<sup>-1</sup>). The activities of SOD, CAT and GPx attained peak values after short exposure time at day 1 or 3 (except for CAT values in digestive gland and GPx value in gill in low Cd concentration). The main antioxidant enzyme in *M. yessoensis* is GPx in gill but CAT in digestive gland and the main detoxification tissue dealing with oxyradicals is digestive gland. These results provide valuable information of Cd accumulation and antioxidant defense mechanism in cadmium-stressed *M. yessoensis* and SOD, CAT, GPx could be useful as biomarkers in predicting heavy metal pollution in scallop aquaculture.

**Key words:** Cadmium, *Mizuhopecten yessoensis*, accumulation, antioxidant enzymes, digestive gland

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### INTRODUCTION

Japanese scallop *Mizuhopecten yessoensis* is a major aquaculture species in North China. By 2005, the scallop production had attained 150,000 metric ton year<sup>-1</sup> (Li *et al.*, 2007). Nevertheless, in recent years, *M. yessoensis* aquaculture has been hampered because of different reasons such as high temperature in Summer or environment factors which were drastically influenced by natural conditions or human behaviors in coastal water (Liu *et al.*, 2010).

Cadmium (Cd) is a ubiquitous heavy metal which spreads in the aquatic surroundings attribute to polluted water, agriculture and industry (Choi *et al.*, 2008). It has severely endangered the environment of the marine and the health of people (Cheng, 2003; Yuan *et al.*, 2004). According to the contaminants investigation in sediments in Bohai bay and the ecological risk assessment from 2001-2005, Cd pollution was the most serious problem in ecology environment (Peng *et al.*, 2009). Also as Vinogradov pointed out, the accumulated heavy metals within the organisms of the marine bivalves were far more than the marine water (Vinogradov, 1959). Moreover, *M. yessoensis*, just as many Pectinidae, intake Cd *in vivo* to higher level than other bivalve mollusks (Boyden, 1977;

Pesch and Stewart, 1980). However, the accumulation of Cd in different tissues of *M. yessoensis* with different cadmium concentrations and exposure times in subacute toxic condition is not very clear until now.

With evolution, organisms have developed several methods to protect themselves by removing the Reactive Oxygen Species (ROS) to prevent cell injury. Antioxidant enzyme system, Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), for example, protects tissues according to directly cleaning up the superoxide and hydrogen peroxide. SOD catalyzes O<sub>2</sub><sup>-</sup> to H<sub>2</sub>O<sub>2</sub> and CAT and GPx convert H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O. Many scientists have explored this mechanism in marine species tissues, especially in the gill and digestive gland. Different responses of these enzymes have been observed when exposed to metallic contaminants, indicating the changes of the enzyme activities depend on the dose and exposing time (Regoli, 1998; Company *et al.*, 2004; Wang *et al.*, 2011). Whereas to date, very little is comprehended about the enzymes changes to subacute cadmium exposure in *M. yessoensis*. In addition, some studies have suggested that the antioxidant enzymes could be used as helpful biomarkers for predicting heavy metal pollution in fish and crustaceans (Almeida *et al.*, 2002; Pan and Zhang, 2006; Kouba *et al.*, 2010) but fewer in scallop.

This study is the first report about cadmium accumulation and antioxidant enzymes response of scallop *M. yessoensis* to subacute Cd exposure. Two organs (gill and digestive gland) were chosen because of their important roles in the contamination exposure. The purpose of the present research was to determine cadmium accumulation and understand enzymatic responses of Japanese scallop to subacute Cd exposure. The results provide new information to the aquaculture of *M. yessoensis* and suggest using anti-oxidative enzymes as monitoring tools in scallop aquaculture to monitor heavy metal pollution.

## MATERIALS AND METHODS

**Experimental scallop:** Adult Japanese scallops (*Mizuhopecten yessoensis*) were collected from Dalian Zhangzidao Fishery Group Corporation (Dalian, Liaoning Province, China) in 2011. Only health scallops with an average size ( $8.730 \pm 0.167$  cm (Mean $\pm$ SD, n = 30), shell height) were chosen. The scallops were maintained in circular seawater at  $16 \pm 1^\circ\text{C}$  and 30‰ salinity which was pumped from the first bathing beach of Qingdao and filtered before experiments and were fed with microalgae spirulina. Half of the seawater was renewed daily and ceaseless aeration was keeping during all the experiment period.

**Experimental design:** Scallops were placed in fiber glass tanks ( $50 \times 40 \times 30$  cm) and were divided into four groups (one control group and three Cd-treatment groups). The  $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$  (Kanto Chemical Co., Tokyo, Japan) was dissolved in seawater to attain  $\text{Cd}^{2+}$  concentrations of 0.05, 0.1 and 0.2  $\text{mg L}^{-1}$  which were 10, 20 and 40 times of the  $\text{Cd}^{2+}$  standard of the seawater based on water quality standard for fisheries of China ( $\text{Cd}^{2+} \leq 0.005$   $\text{mg L}^{-1}$ ). Three replicates were set for each concentration and each one contained 20 scallops. The  $\text{Cd}^{2+}$  concentration of the seawater and spirulina were 0.452  $\mu\text{g L}^{-1}$  and 0.052  $\text{mg kg}^{-1}$  tested by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Elan 6100, Perkin-Elmer, USA) before experiments. The exposure time was 14 days and water was renewed daily then replenished with Cd. No death was founded in every group during the experiment period. On days 0, 1, 3, 6, 10 and 14, gill and digestive gland from randomly selected three individual scallops were sampled and frozen at  $-80^\circ\text{C}$  for further analysis.

**Determination of Cd concentration:** Gill and digestive gland samples from 0, 3, 6, 10 and 14 days were dried at  $60^\circ\text{C}$  for 48 h to constant weight. Dried tissues (about 0.5 g for gill and 0.2 g for digestive gland) were added 3 mL c- $\text{HNO}_3$  in 25 mL Teflon bottles (HP500, CEM, USA). The mixture was placed at room temperature overnight

and the solutions were digested at microwave digestion system (MARS5, CEM, USA) according to setting program. Then, the sample was dissolved in 25 mL pure water. Cd concentrations were tested by inductively coupled plasma-mass spectrometry (ICP-MS, Elan 6100, PE, USA). For quality guarantee, replicate test, control and standard samples (mussel, CRM 278, IRMM, Belgium) were included.

**Biochemical analyses:** For *in vitro* antioxidant enzyme activity measurements, samples obtained from all treatments were homogenized in pre-frozen saline buffer (pH 7.5, 0.0001  $\text{mol L}^{-1}$  EDTA-2Na, 0.01  $\text{mol L}^{-1}$  Tris-HCl, 0.01  $\text{mol L}^{-1}$  Saccharose) and then centrifuged at 10,000 r/min for 20 min at  $4^\circ\text{C}$ . Total protein content in the supernatant was tested according to Lowry's Method (Lowry *et al.*, 1951). The activity of antioxidant enzymes was assayed in triplicate for each sample at the same time using Powerwave XS2 (BioTek, USA).

The activity of SOD was assayed according to the method based on inhibition of cytochrome c reduction at 550 nm for 60 sec (McCord and Fridovich, 1969). The reaction mixture consisted phosphate buffer (50  $\text{mmol L}^{-1}$ , pH 7.8), EDTA (0.1  $\text{mmol L}^{-1}$ ), cytochrome c (10  $\text{mmol L}^{-1}$ ), hypoxanthine (0.05  $\text{mmol L}^{-1}$ ) and the supernatant. The reaction initiated by addition xanthine oxidase (56  $\text{mU mL}^{-1}$ ). One unit SOD was expressed as the enzyme that leads to 50% inhibition of cytochrome reduction (U/mg protein/min).

CAT activity was assayed based on Greenwald (1985). Due to the dismutation of  $\text{H}_2\text{O}_2$ , the activity can be determined by the reduction in absorbance at 240 nm (A240) per minutes. The substrate consisted of 160  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$  (30%) and 100  $\mu\text{L}$  of 0.67  $\text{mol L}^{-1}$  phosphate buffer (pH 7.0). The final reaction system contained 290  $\mu\text{L}$  substrate and 10  $\mu\text{L}$  supernatant or blank. About 3-4 min later, the concentration of  $\text{H}_2\text{O}_2$  was assayed. Results are expressed in units defined as the decrease of 50%  $\text{H}_2\text{O}_2$  per minute (U/mg protein/min).

Gpx activity was assayed based on the method from Xia and Zhu (1987) and Pan and Zhang (2006). The reaction system of the control contained 100  $\mu\text{L}$  of sodium azide phosphate buffer (0.2  $\text{mmol L}^{-1}$  EDTA, 2.5  $\text{mmol L}^{-1}$   $\text{NaN}_3$ , 0.2  $\text{mol L}^{-1}$   $\text{Na}_2\text{HPO}_4$ - $\text{NaH}_2\text{PO}_4$ , pH 7.0) and 100  $\mu\text{L}$  of 1  $\text{mmol L}^{-1}$  GSH and the reaction system of the treatment contained 100  $\mu\text{L}$  of sample and 25  $\mu\text{L}$  of 1  $\text{mmol L}^{-1}$  GSH. These cuvettes were placed at  $37^\circ\text{C}$  for 5 min and then were added with 50  $\mu\text{L}$   $\text{H}_2\text{O}_2$  (1.25  $\text{mmol L}^{-1}$ ). The end of the reaction was determined by adding 1 mL of 50  $\text{g L}^{-1}$  trichloroacetic acid. After keeping in  $37^\circ\text{C}$  for 3 min, the cuvettes were centrifuged at 4,000 r/min for 5 min. Then 100  $\mu\text{L}$  supernatant were taken and added with 125  $\mu\text{L}$   $\text{Na}_2\text{HPO}_4$  (0.4  $\text{mol L}^{-1}$ ) and 25  $\mu\text{L}$  of DTNB (2 mg DTNB, 0.05 g Na-citrate, 5 mL

phosphate buffer). The reaction of coloration was 5 min and then assayed the absorbance at 412 nm. One unit was expressed as 1 nmol GSH reduction in 1 min (U/mg protein/min).

**Statistical analysis:** All data were displayed as Mean±SE (Standard Error) and all the comparison were carried out between control and Cd treatment group. The differences were considered by one-way analysis of variance (one way ANOVA), followed by Least Significant Difference (LSD) test. SPSS analysis software was adopted (V.16.0, Chicago, IL, USA).  $p < 0.05$  were considered as statistically significant difference.

## RESULTS

**Cadmium bioaccumulation:** Changes of Cd bioaccumulation in gill and digestive gland with exposure time and dose were displayed in Fig. 1. The levels of Cd in control group in both tissues maintained stable during experimental period ( $1.8-3.2 \mu\text{g g}^{-1}$  for gill;  $2.41-2.93 \mu\text{g g}^{-1}$  for digestive gland). In the treatment groups, however the amount of Cd increased significantly in all doses with the time. The maximal accumulation was observed at day 10 in gill and day 14 in digestive gland with  $134.20$  and  $109.20 \mu\text{g g}^{-1}$  in the  $0.2 \text{ mg L}^{-1}$  and  $0.1 \text{ mg L}^{-1}$  Cd treatment, respectively and they increased by 52 fold and 36 fold compared to control treatments. However, the levels of Cd in  $0.05 \text{ mg L}^{-1}$  Cd treatment in both tissues displayed relatively mild increase.

In gill, the tendency of Cd accumulation with time in the  $0.1$  and  $0.2 \text{ mg L}^{-1}$  groups was not as liner as the digestive gland. However, the Cd accumulation tendency in gill began to decrease after day 10.

**Antioxidant enzymes activities:** The effects of the Cd exposure on the activities of antioxidant enzymes were shown in Fig. 2. Significant difference ( $p < 0.05$ ) was observed during specific experimental times between treatment and control and the tendency with time

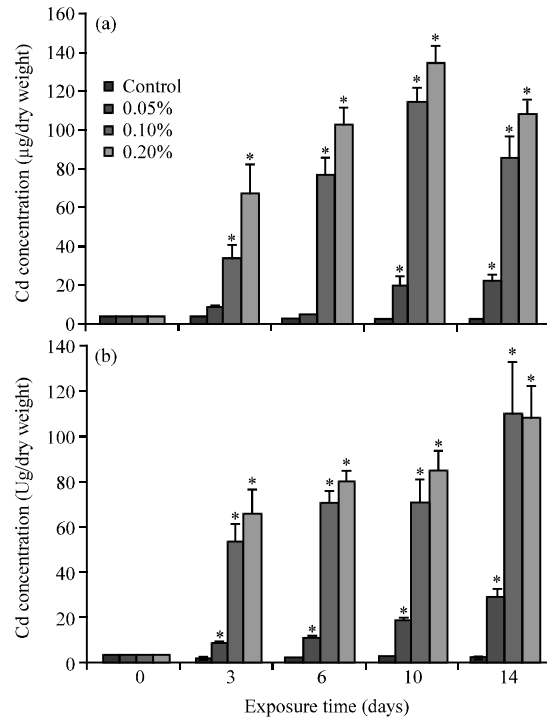


Fig. 1: Bioaccumulation of: a) Cd in gill; b) Digestive gland of *M. yessoensis*; \*Significant differences ( $p < 0.05$ ) between the control and Cd-treatment at different time; values are displayed as Means±SD (n = 3)

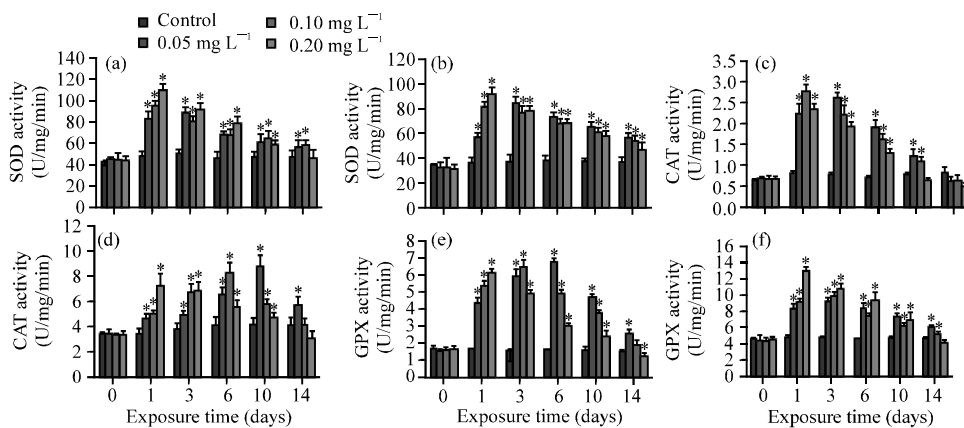


Fig. 2: Effect of Cd exposure (0, 0.05, 0.1, 0.2 mg L<sup>-1</sup>) for 14 days on the activities of: a) SOD in gill; b) Digestive gland; c) CAT in gill; d) Digestive gland; e) GPx in gill; f) Digestive gland of *M. yessoensis*; \*Significant differences ( $p < 0.05$ ) between the control and Cd-treatment at different time; values are displayed as Mean±SD (n = 3)

displayed like a peak roughly speaking. The activities of SOD and CAT in gills in the 0.1 and 0.2 mg L<sup>-1</sup> groups reached the peak at day 1 but those of 0.05 mg L<sup>-1</sup> treatment reached the peak at day 3, followed by a decrease in activities. Moreover, gill CAT activities decreased faster and were inhibited at day 14, although the changes were not significant ( $p > 0.05$ ). GPx activities in gills under different Cd concentrations were shown in Fig. 2. The groups of 0.05, 0.1 and 0.2 mg L<sup>-1</sup> get the peak at the time of day 6, 3 and 1, respectively and the GPx activity of 0.2 mg L<sup>-1</sup> group was significantly inhibited at day 14 ( $p < 0.05$ ).

In digestive gland, the activities of SOD, CAT and GPx for 0.2 mg L<sup>-1</sup> group reached the peak point at day 1 followed by a drop and both CAT and GPx were inhibited at day 14 compared with control. On day 3, the activities of SOD and GPx in 0.05 mg L<sup>-1</sup> group displayed maximum increase and those of the 0.1 mg L<sup>-1</sup> group had the similar tendency, although the SOD activity at day 1 was slightly higher than that at day 3. The CAT values of the 0.05 and 0.1 mg L<sup>-1</sup> treatments increased slowly to the maximum at day 10 and 6 that of the 0.1 mg L<sup>-1</sup> group returned to the control level at day 14.

## DISCUSSION

Many earlier studies have investigated Cd accumulation in marine animals including fish, shellfish and bivalve mollusk in natural or experimental conditions (Morsy and Protasowicki, 1990; McPherson and Brown, 2001; Sokolova *et al.*, 2005; Dang and Wang, 2009). However, the relationships among exposure time, sedimentation and toxicity of metals are not very clear under different exposure conditions (Peakall and Burger, 2003). Also, detoxification ability and distribute of the heavy metal in tissues are often related with different sedimentary rate and time of stress (Reynders *et al.*, 2006). For this reason, present study investigated the Cd accumulation and the response of antioxidant defense system in gill and digestive gland of *M. yessoensis* under experimental conditions via a waterborne approach and demonstrated the relationship between them.

As presenting in Fig. 1, the Cd concentration in both tissues displayed a linear relationship with the exposure doses and had a curvilinear change with time extension. This phenomenon has been observed in earlier studies (Engel and Fowler, 1979; Zaroogian, 1980). The only decrease of Cd accumulation happened in the gill at 0.1 and 0.2 mg L<sup>-1</sup> treatments after 10 days of stress which could attribute to gill mitochondria impairment under the high level of Cd as mitochondria is the main organelle uptaking the dissolved ions in gill (Sokolova *et al.*, 2005).

This could bring serious effect on organism's metabolism because the gill is an important tissue dealing with oxygen intake and multiple energy metabolism processes (Kennedy and Newell, 1996). Meanwhile, the Cd concentration in gill in high level Cd exposure groups (0.1, 0.2 mg L<sup>-1</sup>) were higher than that in digestive gland, supposing that gill could be the key tissue for Cd intake in *M. yessoensis*. Similar conclusions for Cd accumulation have been shown in many other kinds of mollusks (Engel and Fowler, 1979; Ward, 1982; Prakash and Rao, 1995). However, different patterns occurred in some kinds of crabs and mussels (Falconer *et al.*, 1986; Everaarts, 1990; Depledge *et al.*, 1993) in which more Cd were ingested in digestive gland. The main reason for that could be due to the different species variations in accumulation behavior, absorbability or the effect of gonad which can reduce trace metal concentration in digestive gland (Regoli, 1998).

More and more evidences have shown that metabolism of heavy metals play an important role as catalysts in the process of oxidative damage of biomolecules and the toxicities about those metals can cause oxidative tissue damage (Stohs and Bagchi, 1995) producing oxygen free radicals ( $O_2^-$ ,  $H_2O_2 \cdot OH$ ) or reactive oxygen species (Novelli *et al.*, 1998; Martinez-Alvarez *et al.*, 2005). Those can cause serious toxic to aquatic organism and lead to lipid peroxidation in cell nucleotide redox and genotoxicity (Lemaire and Livingstone, 1993; Pan and Zhang, 2006). In order to protect the tissue or organelle against the Cd toxicity, free radical scavengers and antioxidants are induced (Fariss, 1991) like antioxidant enzymes (SOD, CAT, GPx), symbiotic bacteria (Company *et al.*, 2004) and vitamin C (Chandran *et al.*, 2005).

The influences of Cd exposure on the antioxidant defense system have been researched in many species including fishes (Almeida *et al.*, 2002; Basha and Rani, 2003; Atli and Canli, 2010), crustaceans (Pan *et al.*, 2004; Pan and Zhang, 2006) and marine invertebrates (Wang *et al.*, 2011). These studies suggested that antioxidant enzyme activities increased fast in low concentration of Cd exposure while dropped or even be suppressed when the exposure time was prolonged or dosage enhanced. The present study showed similar results, showing the enzyme activities in both tissues reached the peak levels after short exposure time at day 1 or 3 except for CAT activities in digestive gland and GPx activity of 0.05 mg L<sup>-1</sup> in gill. These results indicate an increased capability of organisms to scavenge oxyradicals and ROS in the initial exposure time. However, the subsequent decrease of the activities suggests a decreased ability of the organisms, probably due to the

severe damage of cells with the increased exposure time and Cd accumulation. Meanwhile, some other studies reported different results (Doyotte *et al.*, 1997; Company *et al.*, 2006), displaying that activities of SOD, CAT and GPx were inhibited or remained constant during all experimental period. Therefore, the mechanisms of Cd toxicity remains not completely understood. The responses of the antioxidant system may depend on the species and exposure conditions (e.g., age, dose and time).

In gills of *M. yessoensis*, SOD and CAT activities in high level Cd (0.1, 0.2 mg L<sup>-1</sup>) decreased after 1 day and in low Cd level (0.05 mg L<sup>-1</sup>) decreased after 3 day. However, the GPx activity in 0.05 and 0.1 mg L<sup>-1</sup> treatments increased until 6 and 3 days, respectively. This could be an evidence that Gpx is the main enzyme of scavenging oxyradicals in gill (Pan *et al.*, 2004; Wang *et al.*, 2011). In addition, researchers noted that CAT activities in digestive gland in the 0.05 and 0.1 mg L<sup>-1</sup> treatments were still high until 10 and 6 day, respectively when SOD and GPx activities decreased. This suggests that CAT might play the main role in digestive gland of *M. yessoensis*.

Comparing the antioxidant enzymes activities between the two tissues, SOD in gill was higher than that in digestive gland. This could be the evidence of the function of the gill. Gill are the main tissue for oxygen intake (Sokolova *et al.*, 2005). Therefore, increased oxygen is needed in gill because stress could induce SOD rapidly in short time when the scallop is exposed to Cd. In contrast, the lower SOD activities in digestive gland might attribute to the introduction of MT which combined with -OH much faster than SOD (Li *et al.*, 1996). Moreover, the value of CAT or GPx in gill of *M.yessoensis* is much lower than that in digestive gland in all treatments and all experimental time. These observations are similar to what have been reported in several other researches (Cossu *et al.*, 1997; Wang *et al.*, 2011). These conclude that digestive gland is the major detoxified site of bivalves and sedimentary organ of antitoxic enzyme.

As the results showed, Cd accumulation in both tissues increased with prolonged time and increased doses. However, the antioxidant enzymes did not display the same tendency. The high accumulation of Cd in both tissues may account for this. When the amount of Cd accumulates to a high level, the ceaseless rising of LPO might exceed the compensatory increasing in antioxidant enzymes which would lead to organism damage and do harm to antioxidant enzymes (Asagba *et al.*, 2008). Meanwhile, the induction of MT might compensate for the loss of that and protect the organs from the toxicity of Cd.

## CONCLUSION

In this study, gill of *M. yessoensis* is the major organ of Cd accumulation and digestive gland is the major organ of Cd detoxification. Antioxidant enzymes (SOD, CAT, GPx) play an important role in the detoxification system of *M. yessoensis* and can be used as biomarkers in predicting heavy metal pollution in scallop aquaculture.

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