Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 21(2009) 1598–1606
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Joint effects of cadmium and lead on seedlings of four Chinese cabbage cultivars in northeastern China

XU Zhiqiang[1,2], ZHOU Qixing[1,3,*], LIU Weitao[1,2]

1. Key Laboratory of Terrestrial Ecological Process, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China. E-mail: jackdonlv@126.com
2. Graduate University of the Chinese Academy of Sciences, Beijing 100049, China
3. Key Laboratory of Pollution Processes and Environmental Criteria at Ministry of Education, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China

Received 08 December 2008; revised 15 January 2009; accepted 24 February 2009

Abstract

In northeastern China, large area of vegetable land has been simultaneously polluted by cadmium (Cd) and lead (Pb). Joint effects of Cd and Pb on Chinese cabbage (*Brassica pekinensis* L.) were investigated using the seed germination and sand culture method. Four Chinese cabbage cultivars including Kangbingjinchun (KB), Dongyangchunxia (DY), Qinglvwang (QL) and Qiangshi (QS) from Shenyang in northeastern China were adopted in this study. The results showed that there were positive linear relationships between the inhibitory rate of biomass, root and shoot elongation and the concentrations of Cd and Pb. In particular, root elongation was more sensitive to joint stress of Cd and Pb. The activity of superoxide dismutase and the content of malondialdehyde (MDA), soluble protein (SP) and proline (PRO) changed significantly with increasing exposure concentration of Cd and Pb. The decrement in the activity of antioxidative enzymes, the content of SP and accumulation of MDA were relatively low in KB and QS. PRO played an important role in resisting Cd and Pb stress.

Key words: cadmium; lead; Chinese cabbage; antioxidative enzyme; malondialdehyde; soluble protein, proline
DOI: 10.1016/S1001-0742(08)62461-4

Introduction

With the rapid development of industrial and agricultural production, soil pollution with heavy metals becomes a severe and growing problem. In China, the area of cultivated land contaminated by heavy metals, such as cadmium (Cd), lead (Pb), arsenic (As) and chromium (Cr), is almost 2×10^{11} m^2, and over 1.2×10^7 tons of edible grains are polluted each year (Gu *et al*., 2002). Compared with other agricultural corps, vegetables accumulate heavy metals more easily and abundantly. Investigations on the content of heavy metals in vegetables have been carried out in many cities in China. The results showed that the concentration of some toxic heavy metals in many agricultural products usually exceeded their corresponding national sanitary standard, and Cd and/or Pb are most serious (Li and Zhang, 2008). In particular, large area of vegetable fields in northeastern China is being polluted by Cd and Pb because of sewage irrigation, ore mining and smelting, atmospheric precipitation and application of phosphorus fertilizers. The Zhangshi Irrigation Area in Liaoning Province, northeastern China is one of the most representative Cd and Pb polluted farms (Zhou and Huang, 2001). Chinese cabbage is widely planted in northeastern China. With an increase in metal pollution of vegetable fields, the pollution of Chinese cabbage by heavy metals is being concerned.

In general, many heavy metals including Cd and Pb are nonessential elements with biological toxicity. Cd is one of the most hazardous environmental contaminants (Alloway, 1994). Which can found to induce oxidative stress in cells (Sandalio *et al*., 2001), and can either inhibit or stimulate the activity of several antioxidative enzymes (Corrêa *et al*., 2006). Similarly, Pb can reduce photosynthetic capacity, respiration and nitrogen metabolisms and affect the antioxidant status (Paolacci *et al*., 1997). Moreover, both Cd and Pb can induce the generation of reactive oxygen species (ROS), which unbalances cellular redox, inactivate enzymes, and cause a lipid peroxidation (Moldovan and Moldovan, 2004). The antioxidative enzyme system including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) can protect a plant from oxidative stress. SOD catalyzes the disproportionation of two $O_2^{\bullet-}$ radicals to H_2O_2 and O_2 (Salin, 1988), and POD and CAT participate in the decomposition of H_2O_2. Malondialdehyde (MDA) is a product of damage to membrane lipids in response to toxic chemicals, which can also be used to assess oxidative stress (Jin *et al*., 2002). Proline (PRO) is an organic osmolyte which can accumulate in a variety of

* Corresponding author. E-mail: zhouqx@iae.ac.cn

plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and toxic heavy metals. It can also accelerate ROS scavenging systems. The accumulation of PRO in a plant is thus considered to be physiological adaptation under environmental stresses (Ishitani *et al*., 1995; Stevens *et al*., 1997; Zhu *et al*., 2002). Many plants at seed germination and seedling stage are sensitive to environmental factors. Therefore, the change of plant growth at the germination and seedling stage under heavy metal stress is often regarded as an important index to evaluate plant tolerance to heavy metals (Peralta *et al*., 2001; Abedin and Meharg, 2002).

In the past, effect of single heavy metal on soil-crop systems was extensively investigated (Vallee and Ulmer, 1972; Shah *et al*., 2001; Seregin *et al*., 2003; Yang *et al*., 2006). Although joint effects of some heavy metals on soil-plant systems have been explored (Liu *et al*., 2007; Guo *et al*., 2007; Sun *et al*., 2008), the knowledge about joint effects of Cd and Pb on Chinese cabbage is still scarce. Thus, it is of scientific significance and practice values to investigate joint effects of Cd and Pb on the biomass of vegetable seedlings, the elongation of roots and shoots, the activity of antioxidative enzymes, and the content of MDA and PRO in Chinese cabbage.

1 Materials and methods

1.1 Materials

All reagents used in the study were of analytical grade. The tested forms of Cd and Pb were $CdCl_2 \cdot 2.5H_2O$ and $Pb(NO_3)_2$, which were bought from the Tianjin Kermel Chemical Reagent Co., Ltd., China.

There were four cultivars of Chinese cabbage (*Brassica pekinensis* L.), including Kangbingjinchun (KB), Dongyangchunxia (DY), Qinglvwang (QL) and Qiangshi (QS), which are widely planted in Liaoning Province, northeastern China. They were bought from Shenyang Agricultural University as vegetable seeds.

1.2 Seed germination and metal exposure

Chinese cabbage seeds were surface-sterilized in 3% (*V/V*) H_2O_2 for 15 min and then washed thoroughly with deionized water. The seeds were put into Petri dishes containing filter papers, which were moistened with 3 mL aqueous mixtures of Cd and Pb, and germinated in the dark at 25°C for 4 d. The seeds were exposed to different concentrations of Cd (0, 12, 15, and 18 mg/L) and Pb (0, 15, 20, 25, 30, 35, and 40 mg/L) during germination. The tested Cd or Pb concentrations ranged from 10% to 60% of the inhibitory rate of root elongation, which was determined in the pre-experiment. After 4 d exposure, root length, shoot height and biomass were measured.

1.3 Sand culture

Quartz sands (50% of 0.5–1.0 mm and 50% of 1.0–2.0 mm in diameter) were pretreated with dilute hydrochloric acid, and then washed thoroughly with deionized water. After 500 g of quartz sands were put into a plastic pot, they were watered with 150 mL half-strength Hoagland nutrient solution. The Hoagland nutrient solution was consisted of (mg/L): $Ca(NO_3)_2 \cdot 4H_2O$ 945, KNO_3 607, $NH_4H_2PO_4$ 115, $MgSO_4 \cdot 7H_2O$ 493, $FeSO_4 \cdot 7H_2O$ 13.9, Na_2-EDTA 18.65, H_3BO_3 2.86, $MnSO_4 \cdot 4H_2O$ 2.13, $ZnSO_4 \cdot 7H_2O$ 0.22, $CuSO_4 \cdot 5H_2O$ 0.08, and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.02. The pH of the solution was adjusted to 6.0 by dilute HCl or NaOH solution. The seeds were surface-sterilized in 3% (*V/V*) H_2O_2 as described above, and soaked in deionized water for 36 h. Then 10 uniform seeds were selected and transferred to pots. The plants were cultivated in a growth chamber at 20°C/28°C with 12 h light/12 h dark cycle. One week later (the seedlings had three leaves), the plants were watered with full strength Hoagland nutrient solution. Cd and Pb were added to the pots with full strength Hoagland nutrient solution after 14 days, and the concentrations were selected based on the germination experiment. Then the plants were incubated for another week. The nutrient solution was renewed every 3 days. After total 3 weeks cultivation, the plants were used for biochemical analysis.

1.4 Determination of antioxidative enzyme activity and soluble protein content

About 0.2 g leaf tissues were homogenized in an ice-cooled mortar with 5 mL of 50 mmol/L Na-phosphate buffer (pH 7.8) containing 0.1 mmol/L Na_2-EDTA and 1% (*W/V*) polyvinyl-polypirrolidone (PVP). The extract was centrifuged at 10000 r/min for 15 min at 4°C, and the supernatant was prepared for the determination of soluble protein (SP) content and enzyme activity.

The SP content in the supernatant was determined using the dye-binding method by Bradford (1976). The activity of SOD was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (Stewart and Bewley, 1980). The activity of POD was determined using guaiacol substrates following the method described by Wu and von Tiedemann (2002). The activity of CAT was measured as the decreasing absorbance at 240 nm due to the hydrolysis of H_2O_2 using the method introduced by Aebi (1984).

1.5 Estimation of lipid peroxidation and determination of PRO

Lipid peroxidation was assayed by determining the content of MDA (Kramer *et al*., 1991). About 0.5 g leaf samples were extracted in 80% ethanol (Sharp *et al*., 1990) and PRO was determined using the acid ninhydrin method (Bates *et al*., 1973).

1.6 Statistical analysis

All measurements were replicated four times in independent experiment and the determination of enzyme activity was performed with three parallel samples in all cases. The statistical analyses were performed using the software SPSS (version 11.5). Effects of Cd and Pb on the growth indexes within each cultivar were assessed using two-way ANOVA (Zar, 1999), and the two factors were "Cd concentration" and "Pb concentration". Differences between cultivars were analyzed using one-way ANOVA. The LSR

multiple comparison test was used to analyze data from the germination test and sand culture, respectively, at $P < 0.05$ (Breslow, 1974).

2 Results and discussion

2.1 Joint effects of Cd and Pb on Chinese cabbage at seed germination stage

The variance analysis showed that there was no significant difference in the germination rate of Chinese cabbage seeds between the various treatments by Cd and Pb and controls. The change in inhibitory rate of root and shoot elongation and the biomass of seedlings with increasing exposed concentrations of Cd and Pb are depicted in Fig. 1. There was the significant ($P < 0.01$) linear correlation between the inhibitory rate and the tested concentration of Pb when Cd was added at levels of 0, 12, 15 or 18 mg/L. The corresponding regression equations are listed in Table 1.

The root and shoot elongation of all the cultivars were inhibited under the stress of Pb, while the biomass of seedlings increased, compared with the control. When Cd was added, the inhibitory rate increased markedly. The analysis of two-way ANOVA showed that the inhibitory rate of root length, shoot height and biomass of the four cultivars increased significantly ($P < 0.01$) with increasing concentrations of Cd and Pb. The differences of all three growth indexes (the inhibitory rate between 0, 12, 15 and 18 mg/L Cd added level) were significant ($P < 0.05$) for each cultivar. Only when Pb was higher than 20 mg/L, the differences of the root inhibitory rate between different Pb added levels were significant ($P < 0.05$) for KB, QL and

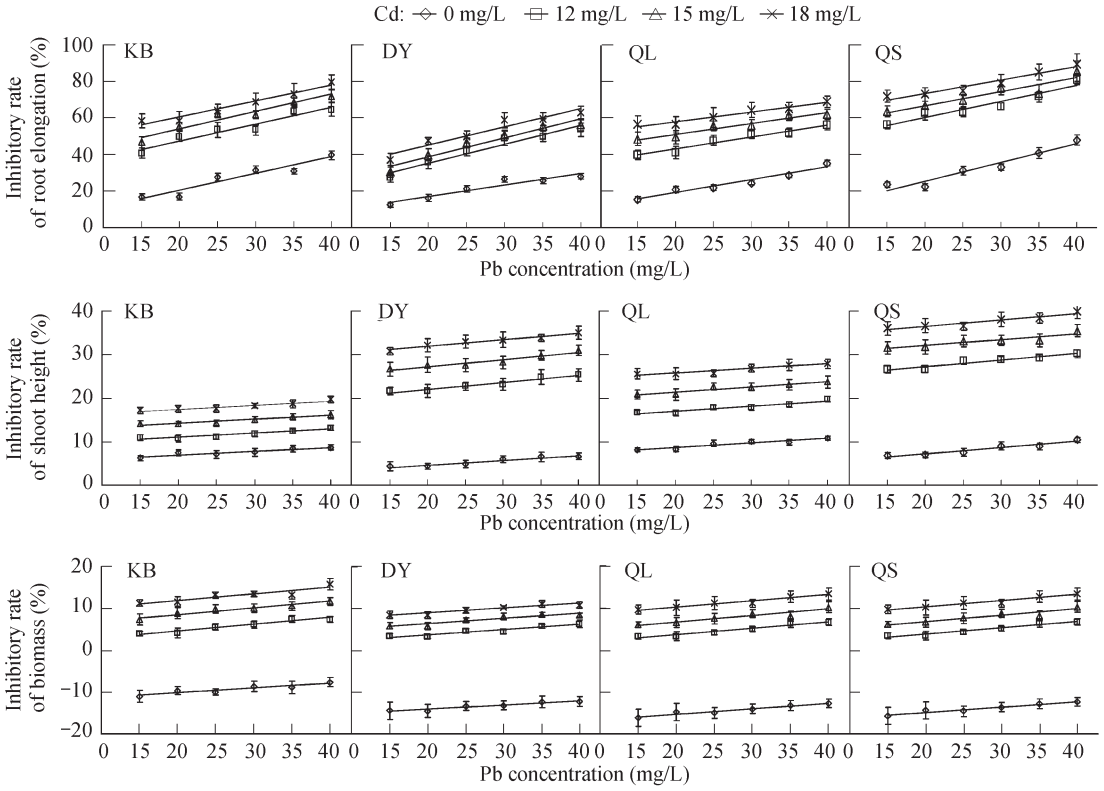

Fig. 1 Inhibitory effects of different Cd and Pb concentrations on the root elongation, shoot height and biomass of Chinese cabbage seedlings. KB: Kangbingjinchun, DY: Dongyangchunxia, QL: Qinglvwang, QS: Qiangshi. Each data point represents mean ± SE.

Table 1 Relationships between the inhibitory rate of root length (RI), shoot height (SI) and biomass (BI) and the concentration of Pb

Cd added (mg/L)		KB		DY		QL		QS	
		Equation	R^2	Equation	R^2	Equation	R^2	Equation	R^2
Root length	0	RI = 0.91x + 2.15	0.912	RI = 0.63x + 4.31	0.913	RI = 0.71x + 5.01	0.950	RI = 1.03x + 4.83	0.937
	12	RI = 0.92x + 29.14	0.963	RI = 1.01x + 14.71	0.945	RI = 0.67x + 29.81	0.958	RI = 0.87x + 42.78	0.920
	15	RI = 0.95x + 35.11	0.942	RI = 1.01x + 18.42	0.941	RI = 0.59x + 39.56	0.943	RI = 0.78x + 50.92	0.870
	18	RI = 0.88x + 42.88	0.927	RI = 0.99x + 24.88	0.925	RI = 0.52x + 47.94	0.946	RI = 0.74x + 58.58	0.936
Shoot height	0	SI = 0.089x + 5.14	0.875	SI = 0.11x + 2.48	0.927	SI = 0.10x + 6.59	0.907	SI = 0.14x + 4.47	0.924
	12	SI = 0.094x + 9.15	0.903	SI = 0.16x + 18.73	0.938	SI = 0.12x + 14.63	0.900	SI = 0.15x + 24.18	0.908
	15	SI = 0.094x + 12.89	0.908	SI = 0.16x + 23.93	0.914	SI = 0.12x + 18.94	0.891	SI = 0.14x + 29.14	0.867
	18	SI = 0.093x + 15.60	0.918	SI = 0.15x + 28.79	0.883	SI = 0.11x + 23.60	0.907	SI = 0.15x + 33.33	0.905
Biomass	0	BI = 0.12x – 12.50	0.871	BI = 0.10x – 16.02	0.925	BI = 0.13x – 17.64	0.934	BI = 0.11x–11.33	0.907
	12	BI = 0.16x + 1.36	0.888	BI = 0.13x + 1.21	0.909	BI = 0.15x + 0.71	0.952	BI = 0.16x + 3.43	0.933
	15	BI = 0.16x + 5.38	0.886	BI = 0.13x + 3.83	0.905	BI = 0.14x + 3.85	0.906	BI = 0.16x + 6.76	0.886
	18	BI = 0.16x + 8.73	0.862	BI = 0.12x + 6.70	0.896	BI = 0.11x + 8.08	0.936	BI = 0.17x + 9.96	0.895

QS. Changes of shoot height and biomass with Pb levels were lower compared with those of root length. However, there was no significant ($P > 0.05$) interactive effect of Cd and Pb on root length, shoot height and biomass of each cultivar.

The biomass of the seedlings increased from 5% to 15% under the stress of Pb (Fig. 1). Similar findings were reported in previous studies for a broad range of grain and vegetable species (Yu *et al*., 2006; Zhu *et al*., 2007; Shentu *et al*., 2008). The possible reason may be that mild metal stress may cause changes in plant hormones that regulate plant growth and development. The contents of indoleacetic acid, gibberellic acid and cytokinin have been found to increase under different types of heavy metal stress (Péter *et al*., 2003; Liu *et al*., 2005; Atici *et al*., 2005). These endogenous phytohormones can stimulate the growth of plant.

Different cultivars of Chinese cabbage had different responses to stress of Cd and Pb. There was a significant ($P < 0.01$) difference between each growth index of studied four cultivars. At the highest Cd and Pb exposed level, the inhibitory rate of root length of KB, DY, QL and QS reached 79.60%, 62.28%, 69.26% and 89.46%, respectively; and the inhibitory rate of biomass was 15.77%, 10.87%, 13.29% and 17.41%, respectively. Thus, the effect of Cd and Pb on root length and biomass of DY was minimal, while there was the most influence for QS. Similarly, the inhibitory rate of shoot growth for KB was at the lowest (19.67%), and that for QS was still maximum (39.70%). According to Table 1, the half-inhibition dose (ID_{50}) of Pb could be calculated and listed in Table 2. ID_{50} of Pb based on shoot height and biomass changes at the same level of added Cd was much higher than that based on root elongation. At the presence of Cd and Pb, ID_{50} of Pb toxic to cultivars was in the sequence: DY > QL > KB > QS for $R_{ID_{50}}$ of root length, KB > QL > DY > QS for $S_{ID_{50}}$ of shoot height, and DY > QL > KB > QS for $B_{ID_{50}}$ of biomass.

Usually, it is difficult to construct a model to describe joint effects of multiple pollutants because the effects are complicated and many factors such as biological species, pollutant forms and methods can affect experimental results. Thus, the changing trend in slope coefficients of the equations in Table 1 is complex. The primary judgment of combined effects of Cd and Pb could be made by the inhibitory rate of the growth indexes. Table 3 shows the inhibitory rate of root length for KB at different added levels of Pb and Cd. Based on the hypothesis that Cd and Pb exerted additive action; each group of the inhibitory rate at different Cd added levels (12, 15, 18 mg/L) was compared with the corresponding hypothetical values. The difference was not significant ($P > 0.05$) at any concentration of Cd. Therefore, it can be concluded that Cd and Pb had an additive action on root elongation for KB. Similarly, the joint effect of Cd and Pb could be judged by the inhibitory rate of root elongation, shoot height and biomass for four studied cultivars (results not shown). Results showed that all the differences were not significant ($P > 0.05$). Thus, Cd and Pb had additive actions on studied Chinese cabbage cultivars at the tested concentrations of Cd and Pb.

Table 2 Half-inhibition dose (ID_{50}) of Pb with different Cd concentrations

Added Cd (mg/L)		0	12	15	18
KB	$R_{ID_{50}}$ (mg/L)	47.64	22.67	15.68	–
	$S_{ID_{50}}$ (g/L)	0.50	0.44	0.39	0.37
	$B_{ID_{50}}$ (g/L)	0.52	0.30	0.28	0.26
DY	$R_{ID_{50}}$ (mg/L)	72.52	34.94	31.26	25.37
	$S_{ID_{50}}$ (g/L)	0.43	0.20	0.16	0.14
	$B_{ID_{50}}$ (g/L)	0.66	0.38	0.36	0.36
QL	$R_{ID_{50}}$ (mg/L)	63.37	30.13	17.69	–
	$S_{ID_{50}}$ (g/L)	0.43	0.29	0.26	0.24
	$B_{ID_{50}}$ (g/L)	0.52	0.33	0.31	0.28
QS	$R_{ID_{50}}$ (mg/L)	43.85	–	–	–
	$S_{ID_{50}}$ (g/L)	0.32	0.17	0.15	0.11
	$B_{ID_{50}}$ (g/L)	0.56	0.29	0.27	0.24

$R_{ID_{50}}$, $S_{ID_{50}}$ and $B_{ID_{50}}$ represent ID_{50} of root length, shoot height and biomass, respectively.

–: when the concentration of Cd was added, the inhibitory rate of root elongation was higher than 50% at whatever concentration of Pb.

There are three established methods for toxic experiments of higher plants, the experiment concerning root elongation, seed germination, and seedling growth at the early development stage (Cheng and Zhou, 2002; Song *et al*., 2002). The determination of growth indexes at seed germination stage is a convenient way to examine the effect of a pollutant on plants. But usually, seed germination is less sensitive compared with root elongation and seedling growth (Peralta *et al*., 2001; Cheng and Zhou, 2002). In our work, the change of seed germination rate was not significant compared with the control when different concentrations of Cd and Pb were applied, which conformed to the above conclusion well. As shown in Fig. 1, the toxicity of Cd and Pb to root and shoot elongation is in the sequence of root elongation > shoot elongation > biomass. Therefore, root is more sensitive to Cd and Pb. This result is consistent with many previous research works (Wang and Zhou, 2005; Song *et al*., 2002). Responses of various cultivars to the same contaminant were different. Table 2 reveals that when Cd was added, $R_{ID_{50}}$ and $B_{ID_{50}}$ of DY are the highest in the corresponding indexes, whereas $R_{ID_{50}}$, $S_{ID_{50}}$ and $B_{ID_{50}}$ of QS are the lowest compared with those of the other cultivars indicating that the tolerance of DY to joint toxicity of Cd and Pb is the highest and the one of QS is the lowest at germination stage.

2.2 Joint effects of Cd and Pb on activity of antioxidative enzymes

The activity of SOD, POD and CAT changed significantly ($P < 0.01$) with an increase in the concentrations of Cd and Pb (Fig. 2). Noticeably, the activity of all enzymes increased, or increased at first and then decreased when the concentrations of Cd and Pb were relatively low. However, when exposed Cd was up to 18 mg/L, the activity of all enzymes, except POD activity of QS, decreased. The activity of SOD and CAT for DY and QL even decreased to nearly half of that for controls. For Pb with or without the addition of Cd, SOD activity in all cultivars changed significantly ($P < 0.01$), and the effects of Cd and Pb interaction on SOD activity was also significant ($P < 0.01$). However, the responses of POD and CAT in various

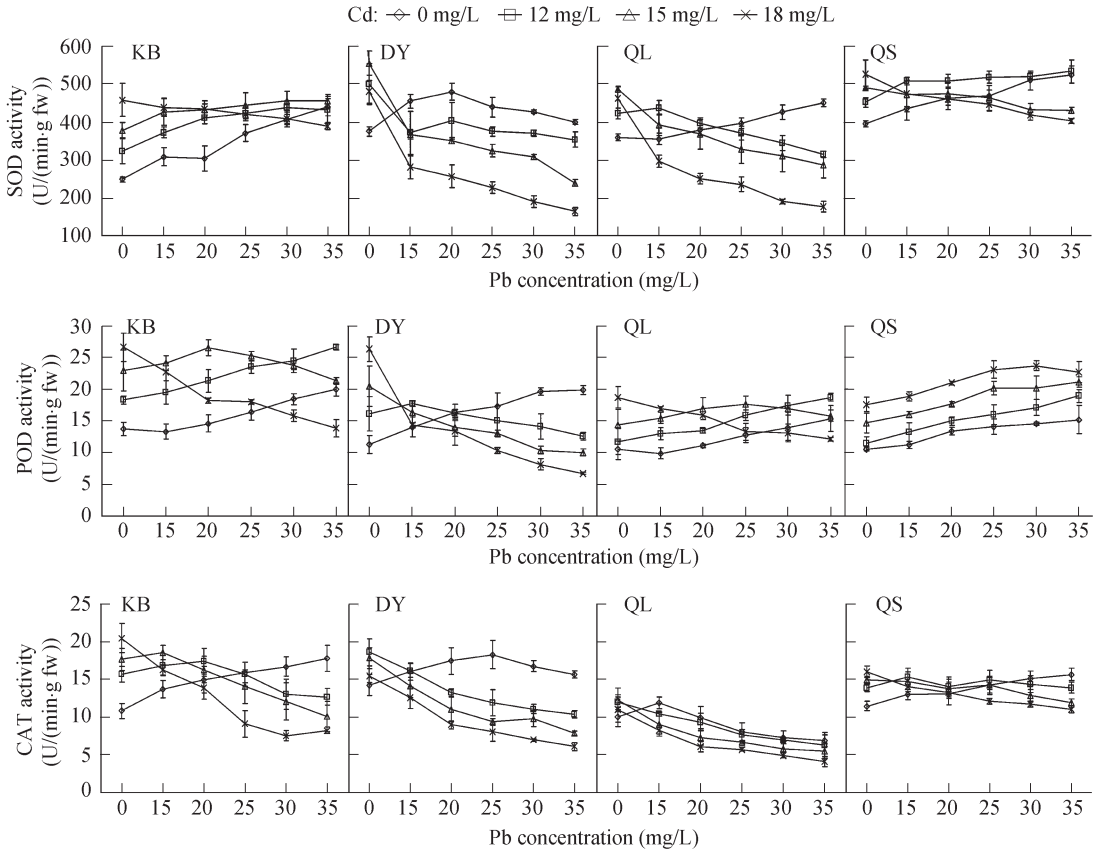

Fig. 2 Joint effects of Cd and Pb on superoxide dismutase (SOD), peroxidase (POD) and catalase activity (CAT). Each data point represents mean ± SE.

Table 3 Inhibitory rate (mean value) of root elongation for KB under different concentrations of Pb and Cd

	0 mg/L Pb	15 mg/L Pb	20 mg/L Pb	25 mg/L Pb	30 mg/L Pb	35 mg/L Pb	40 mg/L Pb
0 mg/L Cd	0	16.85	17.03	27.69	31.70	31.02	39.71
12 mg/L Cd	24.39	40.86	49.88	53.49	53.53	63.65	64.66
15 mg/L Cd	29.14	46.74	55.31	62.23	61.87	68.73	71.88
18 mg/L Cd	33.32	58.49	58.59	63.99	68.65	73.18	79.60

cultivars to single and joint effects of Cd and Pb differed. POD in KB and QL was sensitive to Cd and the interaction of Cd and Pb ($P < 0.01$), but less sensitive to Pb ($P > 0.05$), while it was contrary for DY and QL. CAT was sensitive to Cd and Pb ($P < 0.01$) only for DY. In all cultivars, the interactive effects of Cd and Pb on CAT were not significant ($P > 0.05$). Therefore, SOD was more sensitive to exogenous Cd and Pb than other enzymes. Figure 2 also reveals that the changing degree of SOD activity for KB and QS was lower than that for DY and QL. Although SOD activity of KB and QS also decreased at the presence of high level Cd and Pb, it was still higher than that in controls. It was noticeable that among four cultivars, the changing extent of activity for all enzymes for QS was at the lowest level.

Heavy metals can induce the generation of ROS, such as superoxide radical ($O_2^{\bullet -}$), hydroxyl radical (·OH), hydrogen peroxide (H_2O_2) and alkoxyl radical (RO·) (Bowler *et al*., 1992; Babu *et al*., 2001). ROS can damage membrane lipids, proteins, pigments, and nucleic acids, and plant evolves the antioxidant defense system to prevent the adverse effects of oxidative stress to cells (Jung *et al*., 2000). Inadequate activities of antioxidant defense systems cause oxidative damage in plants exposed to Cd (Metwally *et al*., 2003). The antioxidative enzyme system is one of the protective mechanisms in a plant. The increased activity of antioxidative enzymes in a plant indicated the formation of ROS (Pflugmacher, 2004), and the present study indicated the generation of oxidative stress in Chinese cabbage since all studied enzymes activity increased at low Cd and Pb added levels. But SOD activity in four studied cultivars decreased more or less at high Cd and Pb levels. This phenomenon was also reported in other publications. Gallego (1996) found that SOD activity decreased in sunflower leaves under the stress of 0.5 mmol/L Cd; and reduction of the SOD activity was also observed in wheat leave (Panda *et al*., 2003). Casano (1997) thought oxidative damage occurred if the production rate of the superoxide radical under environmental stress exceeds SOD activity. Similarly, the activity of POD and CAT was inhibited under severe oxidative stress, which fits to pervious studies on heavy metals (Sandalio *et al*., 2001; Fornazier *et al*., 2002; Shim *et al*., 2003). The decrease in the activity of enzymes may reflect serious toxic effects from high levels

of heavy metals. When the environmental stresses exceed the tolerant threshold of a plant, the antioxidative enzyme system is depleted.

2.3 Joint effects of Cd and Pb on the content of MDA, SP and PRO

The changes of MDA, SP and PRO content under Cd and Pb stress are shown in Fig. 3. MDA is an end product of lipid peroxidation, which will accumulate in a plant under environmental stress (Skórzyńska-Polit and Krupa, 2006). As shown in Fig. 3, the changing trend of MDA content in the cultivars was similar to that of the antioxidative enzymes activity. MDA content increased at first. In KB and QS, MDA content tended to be steady and fluctuated slightly when the concentration of Pb was over 20 mg/L and Cd was over 15 mg/L. While in DY and QL, the contents of MDA decreased when Cd concentration was higher than 15 mg/L. The SP content increased when Pb level was lower than 20 mg/L and Cd concentration was lower than 18 mg/L, and then decreased with increasing Cd and Pb concentrations. The PRO content increased rapidly under Cd and Pb stress, and decreased only when Cd concentration was up to 18 mg/L. But this was not the case of QS. Generally speaking, Cd affected MDA, SP and PRO content significantly ($P < 0.05$), while Pb and its interaction with Cd were less significant. Among the four cultivars, QS had the lowest content of MDA and highest content of PRO, while QL had the highest content of MDA and lowest content of PRO. This implied the existence of an inner connection between these two physiological indexes.

Obviously, the increasing extent of MDA content was different in various cultivars. In KB and QS, the MDA content was lower than that in DY and QL, which could be associated with the accumulation of PRO in a plant. PRO is an osmolyte that can provide protection to the enzymes; protect macromolecules against denaturation; confer rigidity to the cell wall; and scavenge hydroxyl radicals (di Toppi and Gabbrielli, 1999). In current work, the content of PRO in KB and QS was 2–3 times higher than that in DY and QL, and the decreasing extent of SP content and SOD activity in KB and QS was relatively low (Figs. 2 and 3). Among four cultivars, changes of enzymes activity in QS were the smallest, which can also be related with the highest accumulation content of PRO in QS. Giannakoula (2008) reported that in aluminum-tolerant maize, PRO content increased significantly while MDA content changed slightly under aluminum stress. Schat (1997) found that when different ecotypes of *Silene vulgaris* were exposed to Cd, the PRO concentration in leaves of the metal-tolerant ecotype was 5–6 times higher than that in the nontolerant ecotype. Therefore, the accumulation of PRO can be probably regarded as one of the characters of Cd and Pb-tolerant Chinese cabbage. As far as this is concerned, the tolerance of QS to joint toxicity of Cd and Pb was quite diverse at various development stages.

SOD is the primary scavenger of $O_2^{\bullet -}$ radicals generated from normal physiological activities and from exposure to oxidative factors. It also probably plays a key role in defending against toxic ROS accumulation (Bowler *et al*., 1992). As mentioned above, MDA is a product of damage to membrane lipids in response to various pollutants, and PRO will accumulate in a plant under environmental stress. In our work, SOD activity increased

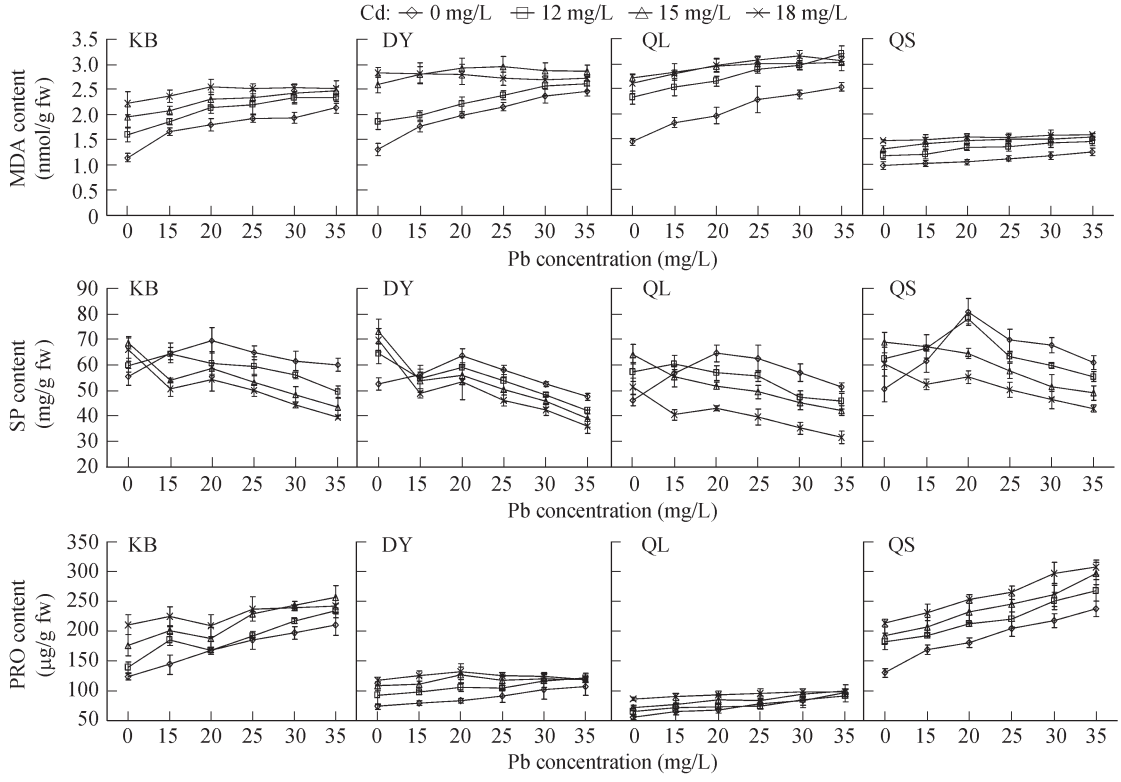

Fig. 3 Joint effects of Cd and Pb on the contents of MDA, SP and PRO. Each data point represents mean ± SE.

when the concentrations of Cd and Pb were low, and the contents of MDA and PRO increased in the same time. That meant the generation of oxidant stress and the startup of the antioxidant defense system. The promotion of SOD activity led to the increase of H_2O_2, consequently the activities of POD and CAT were induced. Even SOD activity decreased along with the increasing concentrations of Cd and Pb, POD activity kept on rising to scavenge H_2O_2. But it seems that CAT was less tolerant to Cd and Pb stress (Fig. 2). At low Cd and Pb levels, the content of SP increased. This may caused by the protection of the antioxidative enzymes and PRO, which alleviated the oxidant stress; or the content of plant hormones which can simulate growth (such as indoleacetic acid) increased at certain concentrations of Cd and Pb (Péter *et al*., 2003; Liu *et al*., 2005; Atici *et al*., 2005). As the analysis above, KB and QS showed more tolerance to Cd and Pb than DY and QL, however, the activity of the antioxidative enzymes and the content of PRO and SP decreased in all cultivars when the concentrations of Cd and Pb were high. Thereby, these physiological processions were all inhibited by high toxic conditions.

It is interesting that the content of MDA decreased at high Cd and Pb added levels. Skórzyńska-Polit and Krupa (2006) and Qiu (2008) also found that the content of MDA decreased in Cd-treated *Phaseolus coccineus* L. and *Arabis paniculata* F. Skórzyńska-Polit and Krupa (2006) considered that this should be attributed to the decomposition of lipid peroxides, or the accumulation of various free fatty acids in stressed plants which change the way of lipid peroxidation. On the other hand, this may be caused by the change of membrane structure, protection of antioxidants (such as glutathione and ascorbate other than PRO) or other physiological processions. Further research is still necessary to understand the specific mechanism.

3 Conclusions

There were significant positive linear relationships between the inhibitory rate of biomass, root and shoot elongation of four Chinese cabbage cultivars and the tested concentration of Pb when Cd was added at the level of 0, 12, 15 or 18 mg/L. Root elongation was the most sensitive growth index reflecting joint stress of Cd and Pb at the germination stage. DY were considered as the most tolerant cultivar to Cd and Pb, whereas QS was the most vulnerable one at germination stage.

The activity of SOD, POD and CAT increased, or increased first and then decreased when the concentrations of Cd and Pb were relatively low. But when Cd was up to 18 mg/L, nearly all the antioxidative enzymes were inhibited. The inhibitory extent for KB and QS was relatively low. SOD was more sensitive to Cd and Pb stress than POD and CAT.

The contents of MDA, SP and PRO changed significantly with the increase in concentrations of Cd and Pb. The content of PRO in KB and QS was 2–3 times higher than that in DY and QL, and the decrease extent of SP content in KB and QS was low. PRO played an important role in resisting the joint stress of Cd and Pb. The accumulation of PRO can be probably regarded as one of the characters of Cd and Pb-tolerant Chinese cabbage.

Acknowledgments

This work was jointly supported by the National Hi-Tech Research and Development Program (863) of China (No. 2006AA06Z386) and the Liaoning Natural Science Foundation (No. 20072018). The authors would also like to thank the Ministry of Education, People's Republic of China for financial support as a grand fostering project (No. 707011), and thank the Sino-Russion Joint Research Center on Natural Resources and Eco-Environmental Sciences, Chinese Academy of Sciences for its partial financial support.

References

Abedin M J, Meharg A A, 2002. Relative toxicity of arsenite and arsenate on germination and early seedling growth of rice (*Oryza sativa* L.). *Plant and Soil*, 243(1): 57–66.

Aebi H, 1984. Catalase *in vitro*. *Methods in Enzymology*, 105: 121–126.

Alloway B J, 1994. Heavy Metals in Soils (2nd ed.). London: Blackie Academic & Professional. 122.

Atici O, Agar G, Battal P, 2005. Changes in phytohormone contents in chickpea seeds germinating under lead or zinc stress. *Biologia Plantarum*, 49(2): 215–222.

Babu T S, Marder J B, Tripuranthakam S, Dixon D G, Greenberg B M, 2001. Synergistic effects of photooxidized polycyclic aromatic hydrocarbon and copper on photosynthesis and plant growth: evidence that *in vivo* formation of reactive oxygen species is a mechanism of copper toxicity. *Environmental Toxicology and Chemistry*, 20: 1351–1358.

Bates L S, Waldren R P, Teare I D, 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil*, 39: 205–207.

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

Breslow N, 1974. Covariance analysis of censored survival data. *Biometrics*, 30: 89–99.

Bowler C, Montagu M V, Inzé D, 1992. Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology*, 43: 83–116.

Casano L M, Gomez L D, Lascano H R, Gonzalez C A, Trippi V S, 1997. Inactivation and degradation of CuZn-SOD by active oxygen species in wheat chloroplasts exposed to photooxidative stress. *Plant and Cell Physiology*, 38(4): 433–440.

Cheng Y, Zhou Q X, 2002. Ecological toxicity of reactive X-3B red dye and cadmium acting on wheat (*Triticum aestivum*). *Journal of Environmental Sciences*, 14(1): 136–140.

Corrêa A X D R, Rörig L R, Verdinelli M A, Cotelle S, Férard J F, Radetski C M, 2006. Cadmium phytotoxicity: quantitative sensitivity relationships between classical endpoints and antioxidative enzyme biomarkers. *Science of the Total Environment*, 357: 120–127.

di Toppi L S, Gabbrielli R, 1999. Response to cadmium in higher plants. *Environmental and Experimental Botany*, 41: 105–130.

Fornazier R F, Ferreira R R, Vitoria A P, Molina S M G, Lea P J, Azevedo R A, 2002. Effects of cadmium on antioxidant enzyme activities in sugar cane. *Biologia Plantarum*, 45: 91–97.

Gallego S M, Benavides M P, Tomaro M L, 1996. Effect of heavy metal ion excess on sunflower leaves: Evidence for involvement of oxidative stress. *Plant Science*, 121(2): 151–159.

Giannakoula A, Moustakas M, Mylona P, Papadakis I, Yupsanis T, 2008. Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. *Journal of Plant Physiology*, 165: 385–396.

Gu J G, Zhou Q X, Wang X, 2003. Reused path of heavy metal pollution in soils and its research advance. *Journal of Basic Science and Engineering*, 11(2): 143–151.

Guo T R, Zhang G P, Zhang Y H, 2007. Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. *Colloids and Surfaces B: Biointerfaces*, 57: 182–188.

Ishitani M, Nakamura T, Han S Y, Takabe T, 1995. Expression of the betaine aldehyde dehydrogenase gene in barley in response to osmotic stress and abscisic acid. *Plant Molecular Biology*, 27(2): 307–315.

Jin P, Ma J M, Yang K J, 2002. Effect of soaking of Hg^{2+} on wheat during its germination and seedling growth. *Journal of Henan Normal University (Natural Science)*, 30(4): 81–84.

Jung S, Kim J S, Cho K Y, Tae G S, Kang B G, 2000. Antioxidant responses of cucumber (*Cucumis sativus*) to photoinhibition and oxidative stress induced by norflurazon under high and low PPFDs. *Plant Science*, 153: 145–154.

Kramer G F, Norman H A, Krizek D T, Mirecki R M, 1991. Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochemistry*, 30: 2101–2108.

Liu S C, Xiao L T, Liao B H, Lu X D, Zhao W K, Kuang F C, Tong J H, 2005. Effects of lead stress on dynamic accumulation of endogenous phytohormones in cucumber seedlings. *Journal of Hunan Agricultural University*, 31(5): 510–513.

Li X, Zhang Y, 2008. The status and general laws of heavy metal pollution of vegetables and farm soil in China. *Sichuan Environment*, 27(2): 94–97, 101.

Liu X L, Zhang S Z, Shan X Q, Christie P, 2007. Combined toxicity of cadmium and arsenate to wheat seedlings and plant uptake and antioxidative enzyme responses to cadmium and arsenate co-contamination. *Ecotoxicology and Environmental Safety*, 68: 305–313.

Metwally A, Finkemeier I, Georgi M, Dietz K J, 2003. Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiology*, 132: 272–281.

Moldovan L, Moldovan N I, 2004. Oxygen free radicals and redox biology of organelles. *Histochemistry and Cell Biology*, 122: 395–412.

Panda S K, Chaudhury I, Khan M H, 2003. Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves. *Biologia Plantarum*, 46(2): 289–294.

Paolacci A R, Badiani M, Damnibale A, Fusari A, Matteucci G, 1997. Antioxidants and photosynthesis in the leaves of *Triticum durum* Desf seedlings acclimated to non-stressing high temperature. *Journal of Plant Physiology*, 150: 381–387.

Peralta J R, Gardea-Torresdey J L, Tiemann K J, Gomez E, Arteaga S, Rascon E, Parsons J G, 2001. Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). *Bulletin of Environmental Contamination and Toxicology*, 66(6): 727–734.

Péter N, Károly B, László G, 2003. Characterization of the stimulating effect of low-dose stressors in maize and bean seedlings. *Journal of Plant Physiology*, 160: 1175–1183.

Pflugmacher S, 2004. Promotion of oxidative stress in the aquatic macrophyte *Ceratophyllum demersum* during biotransformation of the cyanobacterial toxin microcystin-LR. *Aquatic Toxicology*, 70: 169–178.

Qiu R L, Zhao X, Tang Y T, Yu F M, Hu P J, 2008. Antioxidative response to Cd in a newly discovered cadmium hyperaccumulator, *Arabis paniculata* F. *Chemosphere*, 74: 6–12.

Salin M L, 1988. Toxic oxygen species and protective systems of the chloroplast. *Plant Physiology*, 72: 681–689.

Sandalio L M, Dalurzo H C, Gomez M, Romero-Puertas M C, del Rio L A, 2001. Cadmium-induced changes in the growth and oxidative metabolism of pea plants. *Journal of Experimental Botany*, 52: 2115–2126.

Schat H, Sharma S S, Vooijs R, 1997. Heavy metal-induced accumulation of free proline in a metal-tolerant and a non-tolerant ecotype of *Silene vulgaris*. *Physiologia Plantarum*, 101(3): 477–482.

Seregin I V, Kozhevnikova A D, Kazyumina E M, Ivanov V B, 2003. Nickel toxicity and distribution in maize roots. *Russian Journal of Plant Physiology*, 50(5): 711–717.

Shah K, Kumar R G, Verma S, Dubey R S, 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Science*, 161(6): 1135–1144.

Sharp R E, Hsiao T C, Silk W K, 1990. Growth of the maize primary root at low water potentials. II. Role of growth and deposition of hexose and potassium in osmotic adjustment. *Plant Physiology*, 93: 1337–1346.

Shentu J L, He Z L, Yang X E, Li T Q, 2008. Accumulation properties of cadmium in a selected vegetable-rotation system of southeastern China. *Journal of Agricultural and Food Chemistry*, 56: 6382–6388.

Shim I S, Momose Y, Yamamoto A, Kim D W, Usui K, 2003. Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plants. *Journal of Plant Growth Regulation*, 39: 285–292.

Skórzyńska-Polit E, Krupa Z, 2006. Lipid peroxidation in cadmium-treated *Phaseolus coccineus* plants. *Archives of Environmental Contamination and Toxicology*, 50(4): 482–487.

Song Y F, Zhou Q X, Xu H X, Ren L P, Gong P, 2002. Ecological toxicity of heavy metals in soils acting on seed germination and root elongation of wheat. *Chinese Journal of Applied Ecology*, 13 (4): 459–462.

Stevens R G, Creissen G P, Mullineaux P M, 1997. Cloning and characterisation of a cytosolic glutathione reductase cDNA from pea (*Pisum sativum* L.) and its expression in response to stress. *Plant Molecular Biology*, 35(5): 641–654.

Stewart R C, Bewley J D, 1980. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiology*, 65: 245–248.

Sun Y B, Zhou Q X, Diao C Y, 2008. Effects of cadmium and arsenic on growth and metal accumulation of Cd-hyperaccumulator *Solanum nigrum* L. *Bioresource Technology*, 99: 1103–1110.

Vallee B L, Ulmer D D, 1972. Biochemical effects of mercury, cadmium, and lead. *Annual Review of Biochemistry*, 41(10):

91–128.

Wang M E, Zhou Q X, 2005. Single and joint toxicity of chlorimuron-ethyl, cadmium, and copper acting on wheat *Triticum aestivum*. *Ecotoxicology and Environmental Safety*, 60: 169–175.

Wu X Y, von Tiedemann A, 2002. Impact of fungicides on active oxygen species and antioxidant enzymes in spring barley (*Hordeum vulgare* L.) exposed to ozone. *Environmental Pollution*, 116: 37–47.

Yang Z X, Liu S Q, Zheng D W, Feng S D, 2006. Effects of cadmium, zinc and lead on soil enzyme activities. *Journal of Environmental Sciences*, 18(6): 1135–1141.

Yu H, Wang J L, Fang W, Yuan J G, Yang Z Y, 2006. Cadmium accumulation in different rice cultivars and screening for pollution-safe cultivars of rice. *Science of the Total Environment*, 370: 302–309.

Zar J H, 1999. Biostatistical Analysis (4th ed.). New Jersey: Prentice Hall. 123.

Zhou Q X, Huang G H, 2001. Environmental Biogeochemistry and Global Environmental Changes. Beijing: Science Press. 144.

Zhu J K, 2002. Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology*, 53: 247–273.

Zhu Y, Yu H, Wang J, Fang W, Yuan J G, Yang Z Y, 2007. Heavy metal accumulation of 24 asparagus bean cultivars grown in soil contaminated with Cd alone and with multiple metals (Cd, Pb, and Zn). *Journal of Agricultural and Food Chemistry*, 55: 1045–1052.