African Journal of Biotechnology Vol. 11(22), pp. 6091-6099, 15 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2804
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of cadmium stress on growth, metal accumulation and organic acids of *Spartina alterniflora* Loisel.

Min-Wei Chai[1], Rui-Li Li[2]*, Fu-Chen Shi[1]*, Fu-Chun Liu[3], Xiu Pan[1], Di Cao[1] and Xue Wen[1]

[1]College of Life Sciences, Nankai University, Weijin Road 94, Tianjin 300071, China.
[2]School of Environment and Energy, Shenzhen Graduate School of Peking University, Shenzhen 518055, China.
[3]College of Life Sciences, Cangzhou Normal University, Cangzhou 061001, China.

Accepted 1 December, 2011

A study quantifying the effects of exogenous cadmium (Cd) on growth, Cd bioaccumulation and organic acids of *Spartina alterniflora* was conducted. The experiment consisted of three levels of exogenous Cd^{2+} concentrations: 0, 50, and 200 mg/kg. Total Cd and water-soluble Cd were determined. Plant height, tiller number, spike number, electrolyte leakage rate (ELR), free proline, malondialdehyde (MDA), soluble sugar and organic acids were also measured. The results showed that plant height, tiller number and spike number reduced with increasing Cd^{2+} concentration. However, ELR, free proline, MDA, and soluble sugar were all promoted under Cd stress. Cd accumulated in inflorescences, leaves, stems, rhizomes and fine roots, and increased greatly with increasing Cd^{2+} concentration. Exogenous Cd^{2+} significantly reduced the growth of inflorescences, leaves, stems and rhizomes, with Cd accumulations not more than 15.68 µg/g dry weight (DW), respectively. However, biomass of fine roots did not reduce significantly with increasing Cd^{2+} concentration. Furthermore, Cd accumulation in fine roots reached to 390 µg/g DW under the treatment Cd200. Oxalic and citric acids were the two most abundant organic acids in *S. alterniflora*. Contents of oxalic acid in inflorescences, stems and fine roots were all positively related with Cd bioaccumulations indicating that oxalic acid might be related with Cd sequestration in fine roots and Cd transportation from underground to aboveground parts of *S. alterniflora*. Contents of citric acid in fine roots and ascorbic acid in rhizomes increased with increasing exogenous Cd^{2+} concentration, suggesting that accumulation of citric acids in fine roots and ascorbic acid in rhizomes of *S. alterniflora* might also be related to its Cd bioaccumulation and detoxification.

Key words: *Spartina alterniflora*, cadmium pollution, growth, organic acid, phytoremediation.

INTRODUCTION

Estuary wetlands as we all know offer the greatest ecosystem services per unit area compared with other ecosystems (Costanza et al., 1997). Recently, due to anthropogenic activities such as utilization of fertilizer, sewage discharge, energy and fuel production, many hazardous pollutions (including heavy metals) have been released into rivers and lakes (Cuong et al., 2005; Defew et al., 2005). Therefore, salt marsh and seaside sediments on estuaries tend to act as sinks for the derived heavy metals, which cause direct or potential damage to estuary wetland ecosystems (Weis and Weis, 2004). Compared with other heavy metals, cadmium (Cd) in the soil was easier to be absorbed by plant that caused long term potential hazards to human and animal health by food chains (Perronnet et al., 2000; Zhou and Song, 2004). Consequently, it is necessary to remediate the Cd-polluted estuary salt marsh.

Being different from physicochemical and engineering techniques, phytoremediation was a cost-effective and environment friendly technique suitable for large areas of

*Corresponding author. E-mail: fchshi@nankai.edu.cn, liruili@pkusz.ed.cn. Tel: 86-22-23502477, 86-755-26033141. Fax: 86-22-23508800, 8755-26032078.

heavy contaminated soil (Schwitzguébel et al., 2009). Phytoremediation is defined as application of plants to degrade, extract, contain, or immobilize contaminants from soil and water, including phytoextraction and phytostabilization (Manousaki et al., 2009; Schwitzguébel et al., 2009). The former is an effective strategy which transfer and accumulate heavy metals from soil and underground parts of plant into the aboveground parts, and alleviate heavy metal pollution by harvesting its aerial parts (Wei et al., 2008). Phytostabilization means that stabilization of the contaminants (including heavy metals) in plant's rhizosphere which prevents transferring of heavy metals into its aerial parts or leaching into ground water (Dary et al., 2010). Over the past few years, several heavy metal tolerant plant species have been found to be Cd-hyperaccumulators, including *Thlaspi caerulescens* and *Eulaliopsis binata* (Lombi et al., 2000; Liu et al., 2011). However, these species are not suitable for phytoremediation of heavy metal polluted estuary salt marshes because of their small biomass and/or low tolerance to stress factors, such as salinity, water-logging and high pH.

Spartina alterniflora, a kind of halophytes in Atlantic coast, is spreading in many coastal areas all over the world for its powerful reproductive capacity and adaptation to complex environments. In 1979, *S. alterniflora* was introduced to the east coast of China to promote sediment accretion for its significant advantages in protecting seawall, and accelerating sediment deposition (Chung, 2006). So far, *S. alterniflora* have occupied the naked muddy beach and formed dense vegetative colonies along shorelines and inter-tidal flats in estuary wetlands of north China. The rapid spreading of this species into new habitat is at least partly related to its vegetative propagation, which produced new stems from an extensive network system of underground roots. Recently, the phytoremediation of *S. alterniflora* in heavy metal contaminated soil and water has not been reported widely. *S. alterniflora* was a kind of plant that could be used to recover the ecological function of wetland ecosystem seriously polluted by lead (Pb) and Chromium (Cr) in Wenzhou, China (Zhu et al., 2010). Salla et al. (2011) investigated *S. alterniflora* for phytoextraction of selected heavy metals in soils from Southwest Louisiana, and found that *S. alterniflora* exhibited potential for phytoremediation of heavy metals, including chromium (Cr), lead (Pb), iron (Fe) and zinc (Zn). In addition, metal excretion has been observed in *S.alterniflora* through specialized glands or glandular trichomes (Weis and Weis, 2004).

Once stressed by heavy metal, a number of physiological responses in plants were involved, such as production of MDA, damage of cell membrane, accumulation of proline, soluble sugar and heavy metal chelations (organic acids, phytochelatins and metallothioneins) (Zhang and Huang, 2000; Yang et al., 2005). Generally speaking, organic acids chelating with heavy metals in plants or rhizosphere regions mainly included oxalic, malic, and citric acids (Kramer et al., 1996). Organic acids combined with the undesirable heavy metals, reduced the combination opportunities of heavy metals with cellular proteins and enzymes, alleviating the damage caused by heavy metals (Zhu et al., 2006). In addition, organic acids took part in heavy metal absorption, transfer and accumulation in plants (Sun et al., 2011). Most of Cd accumulated in mature and senescent leaves of *Thlaspi caerulescens* was bond by some weak oxygen ligands including organic acids (Küpper et al., 1999). In aboveground part of *Thlaspi goesingense*, nickel (Ni) - citric complex was mainly allocated into cell vacuole under Ni stress (Kramer et al., 2000). Malic acid in leaves of *T. caerulescens* was chelated with Cd in the form of Cd-malate complex, decreasing the subsequent Cd^{2+} efflux to the cytoplasm (Ueno et al., 2005). Both of acetic and citric acids in leaves of *Solanum nigrum* were positively correlated with Cd accumulation (Sun et al., 2006). In addition, the Cd stress could induce the synthesis of acetic acid in leaves of *Rorippa globosa* and *Rorippa islandica*, suggesting that acetic acid might be related to Cd bioaccumulation (Sun et al., 2011).

Based on the discussion above, we hypothesized that Cd may affect Cd bioaccumulation in different tissues of *S. alterniflora*, and change accumulation of organic acids. Therefore, the main purposes of this experiment were (1) to quantify Cd uptake and bioaccumulation in different tissues of *S. alterniflora* seedlings; (2) to test the effects of Cd on growth of *S. alterniflora* seedlings; (3) to analyze changes of organic acids in response to Cd stress, and assess role of organic acids in Cd bioaccumulation and detoxification.

MATERIALS AND METHODS

Plant material and growth conditions

Newly emerged culms of *S. alterniflora* were collected from seashore (117°45′E, 39°03′N) of Tianjin, the middle east province of China in May 2010. Uniform seedlings were transplanted into plastic pots (30 cm diameter × 10 cm depth) filled with 2×10^3 g clean vermiculite. Three seedlings were planted per pot. There were three Cd-level treatments: control (no Cd addition, Cd0), 50 mg/kg (Cu50), and 200 mg/kg (Cu200). Each treatment was performed in four replicate. Cd was applied as $CdCl_2\cdot2.5H_2O$, mixed thoroughly with the vermiculite samples. All the pots were placed outside (day temperature 22 to 28°C, night temperature 16 to 22°C) and protected from the rain. Seedlings were carefully irrigated with Hoagland's nutrient solution every 4 days. Water was added as necessary to maintain the water level at the vermiculite. The experiment was terminated in October when samples of plants were collected and analyzed.

Plant growth and physiological measurements

The plant height, tiller and spike number per plant were measured. Middle leaves (bottom four to five) of *S. alterniflora* were picked up to determine the membrane permeability, free proline, soluble sugar, and MDA, respectively. Membrane permeability can be reflected by electrolyte leakage rate (ELR). ELR was measured with the method described by Lutts et al. (1996). Proline content was determined using ninhydrin colorimetry (Hao et al., 2004). In addition, soluble sugar and MDA were determined using TBA colorimetry (Hao et al., 2004).

Organic acids were measured as follows: a total of 0.5 g fresh weight of tissues (inflorescences, leaves, stems, rhizomes and fine roots) was ground with 10 ml of 0.01mol/l K_2HPO_4-PBS (pH 2.5). After being placed in a water bath at 75°C for 45 min, the homogenates were centrifuged at 3000 rpm, and the supernatants obtained were filtered through a 0.45 µm Millipore filter and used for chromatographic analysis. Organic acids were analyzed by high performance liquid chromatograph (HPLC) as described by Cawthray (2003) and are summaried here; samples were analyzed using a CoMetro 6000 Series with C_{18} column (4.6×250 mm, 5 µm). The column was operated at 30°C. The mobile phase was 97% 0.01mol/l K_2HPO_4-PBS (pH 2.5) added with 3% methanol with a flow rate of 1 ml/min. Organic acids were determined using a Co-Metro 6000 series UV-spectroscopy detector at 210 nm.

Plants were harvested by cutting the shoots at the vermiculite surface and the roots were carefully separated from the vermiculite. Shoots and roots were rinsed with distilled water. Inflorescences, leaves, stems, rhizomes and fine roots were separately dried at 105°C for 30 min, then at 85°C until a constant weight was reached, and dry matter were weighed.

Cd determination

Dried plants were ground using a ball mill, then digested with $HNO_3/HClO_4$ (10:1[v/v]) for determining Cd content. Water soluble Cd in *S. alterniflora* was measured with 0.1 g dried plant powder suspended in 10 ml deionized water following the method of Perronnet et al. (2000). The water extract were centrifuged at 8000 rpm. Then, the supernatant was filtered through a 0.45 µm Millipore filter before determination. Cd content was determined using atomic absorption spectrophotometer (Z-6100; Hitachi, Japan).

Statistical analysis

Each treatment was replicated 3 times for statistical validity. One-way ANOVA was performed for all data sets. Differences among treatments were analyzed taking $P \leq 0.05$ as significant according to Tukey's test. Pearson correlation coefficients were calculated between different parameters.

RESULTS

Plant growth

Growth characteristics of *S. alterniflora* are shown in Table 1. Under treatment Cd200, plant height, tiller and spike number decreased significantly compared with the control ($P<0.05$). However, ELR, MDA and soluble sugar were all significantly promoted ($P<0.05$). In addition, compared with the control, proline did not change significantly under treatment Cd50 initially, but then increased significantly under treatment Cd200 ($P<0.05$).

Plant biomass and Cd accumulation

As shown in Figure 1, inflorescences, leaves, stems, and rhizomes were all significantly inhibited by Cd stress compared with the control ($P<0.05$), with no apparent difference between the treatments of Cd50 and Cd200. Furthermore, biomasses of inflorescences, leaves, stems and rhizomes were reduced by 43.6 to 80.9%, 34.7 to 41.0%, 37.4 to 47.9% and 64.5 to 69.1%, respectively. However, under Cd stress, biomass of fine roots was not reduced greatly compared with the control. On the whole, the biomass sequence of various tissues under the same Cd treatment was fine roots > leaves > stems > rhizomes > inflorescences.

Total Cd contents in inflorescences, leaves, stems, rhizomes and fine roots are shown in Table 2. *S. alterniflora* accumulated most of Cd in fine roots. Particularly, total Cd content in fine roots was 390 µg/g DW under treatment Cd200. In addition, the sequence of total Cd contents in different tissues was fine roots > inflorescences > rhizomes > stems > leaves. The addition of Cd^{2+} also induced increases of water soluble Cd content (Table 2). Moreover, the sequence of contents of water-soluble Cd was fine roots > rhizomes > leaves > stems > inflorescences.

Water-soluble Cd / total Cd in fine roots increased obviously with increasing Cd^{2+} concentration ($P<0.05$). However, in inflorescences and leaves, water soluble Cd / total Cd did not change significantly between the treatments of Cd50 and Cd200. In addition, under the treatment Cd50, water-soluble Cd / total Cd in rhizomes and stems were 39.23 and 31.54%, obviously higher than those under the treatment Cd200, respectively ($P < 0.05$).

Organic acids

Organic acids in different tissues of *S. alterniflora* are shown in Figure 2. Oxalic and citric acids were the most two abundant organic acids in inflorescences, leaves, stems and rhizomes. With increasing Cd^{2+} concentration, oxalic acid contents increased in inflorescences, stems and fine roots, but decreased in leaves and rhizomes. Citric acid content in fine roots was promoted with increasing Cd^{2+} concentration. However, in inflorescences, leaves, stems and rhizomes, citric acid content was negatively affected by Cd stress. As for ascorbic acid, its contents increased in leaves and rhizomes, while decreased in fine roots, with increasing Cd^{2+} concentration. In addition, fumaric acid is the least abundant organic acid detected. Under Cd stress, fumaric acid content decreased in leaves and rhizomes.

Organic acids in relation to Cd bioaccumulation

In aboveground parts (inflorescences, leaves and stems), there were positive linear correlations between oxalic acid contents and Cd bioaccumulations in inflorescences and stems (Table 3). However, citric acid contents were negatively related with Cd bioaccumulations in inflorescences and stems. In addition, positive correlations were found between total Cd and water-soluble Cd bioaccumulations in inflorescences ($r = 0.968^{**}$), leaves ($r = 0.956^{**}$) and stems ($r = 0.975^{**}$).

Table 1. Growth characteristics of *Spartina alterniflora* under different Cd treatment.

Cd treatments (mg/kg)	Height (cm)	Tillers/plant	Spikes/plant	ELR (100%)	Proline (μg/g FW)	MDA (μmol/g FW)	Soluble sugar(mmol/g FW)
0	134.44±9.37[a]	13.89±1.35[a]	3.00±0.33[a]	3.08±0.10[a]	91.50± 6.84[a]	7.22±1.04[a]	85.40± 4.78[a]
50	115.56±8.10[b]	12.33±1.00[ab]	2.11±0.69[ab]	4.40±0.26[a]	94.90± 4.19[a]	10.12±1.78[a]	102.32± 7.76[a]
200	111.11±6.01[b]	10.44±0.38[b]	1.22±0.19[b]	23.68±3.23[b]	142.86±20.48[b]	25.03±3.41[b]	293.92±10.50[b]

The values in the same column followed by the same letters are not significantly different, whereas by the different letters are significantly different at $P < 0.05$.
ELR: Leaf electrolyte leakage rate; MDA: malondialdehyde; FW: fresh weight.

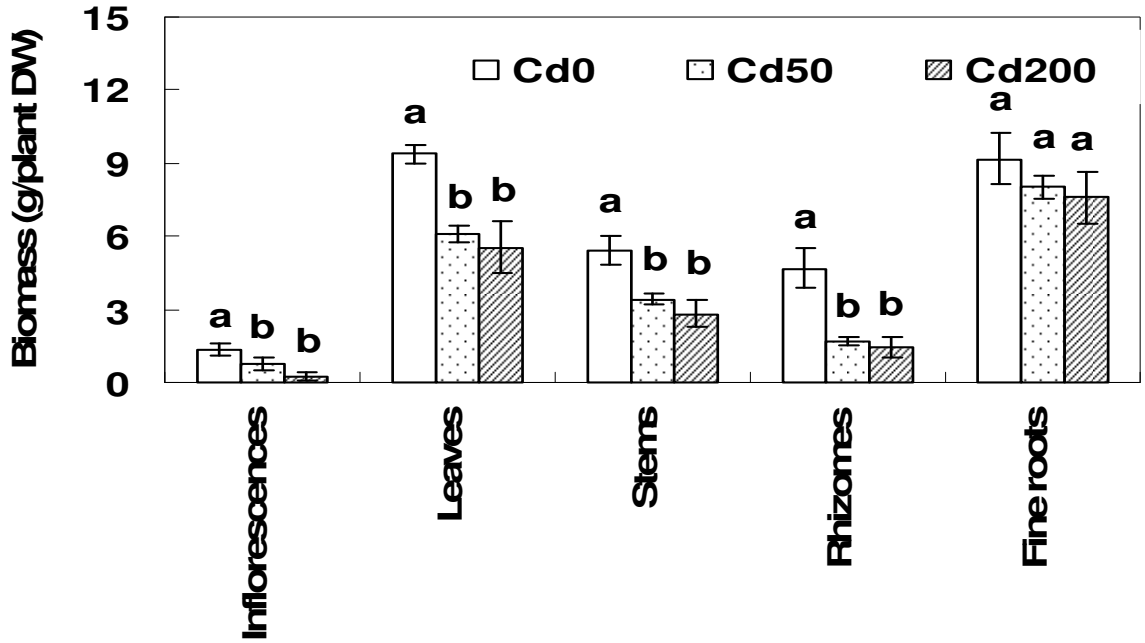

Figure 1. Biomass of inflorescences, leaves, stems, rhizomes and fine roots of *Spartina alterniflora* under different Cd treatments. Values at each tissue having a different small letter are significantly different from each other (p<0.05). Bars denote standard deviation. Cd0～Cd200: 0, 50, 200 mg/kg Cd^{2+} concentrations.

In underground parts (rhizomes and fine roots), ascorbic acid contents were positively related with Cd bioaccumulations in rhizomes. Contents of oxalic, citric and fumaric acids were negatively related with Cd bioaccumulations in rhizomes. In fine roots, contents of oxalic and citric acids were positively related with Cd bioaccumulations, with negative correlations found between ascorbic acid content and Cd bioaccumulation. In addition, there

Table 2. Total Cd, Water-soluble Cd and Percentages of the total Cd that was water-soluble in tissues of *Spartina alterniflora* under different Cd treatment.

Parameter	Cd treatments (mg / kg)	Inflorescences	Leaves	Stems	Rhizomes	Fine roots
Total Cd (μg/g DW)	0	0.92±0.03[a]	1.46±0.10[a]	1.29±0.11[a]	1.29±0.08[a]	2.49± 0.24[a]
	50	11.78±1.30[b]	4.45±0.55[b]	5.34±0.53[b]	5.79±0.41[b]	297.07±12.53[b]
	200	15.68±1.23[c]	5.69±0.72[c]	7.31±0.27[c]	12.05±1.37[c]	390.00± 9.08[c]
Water-soluble Cd (μg/g DW)	0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00± 0.00[a]
	50	1.13±0.19[b]	1.91±0.25[b]	1.68±0.16[b]	2.28±0.28[b]	68.13± 2.72[b]
	200	1.49±0.27[b]	2.78±0.15[c]	1.99±0.10[c]	3.26±0.44[c]	126.70± 5.81[c]
Water-soluble Cd / total Cd (100%)	0	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00± 0.00[a]
	50	9.51±0.63[b]	43.03±3.02[b]	31.54±0.69[c]	39.23±2.44[c]	22.94± 0.24[b]
	200	9.42±0.97[b]	49.19±4.24[b]	27.15±0.67[b]	27.07±1.96[b]	32.48± 1.09[c]

The values in the same tissue and column followed by the same letters are not significantly different, whereas by the different letters are significantly different at $P < 0.05$.

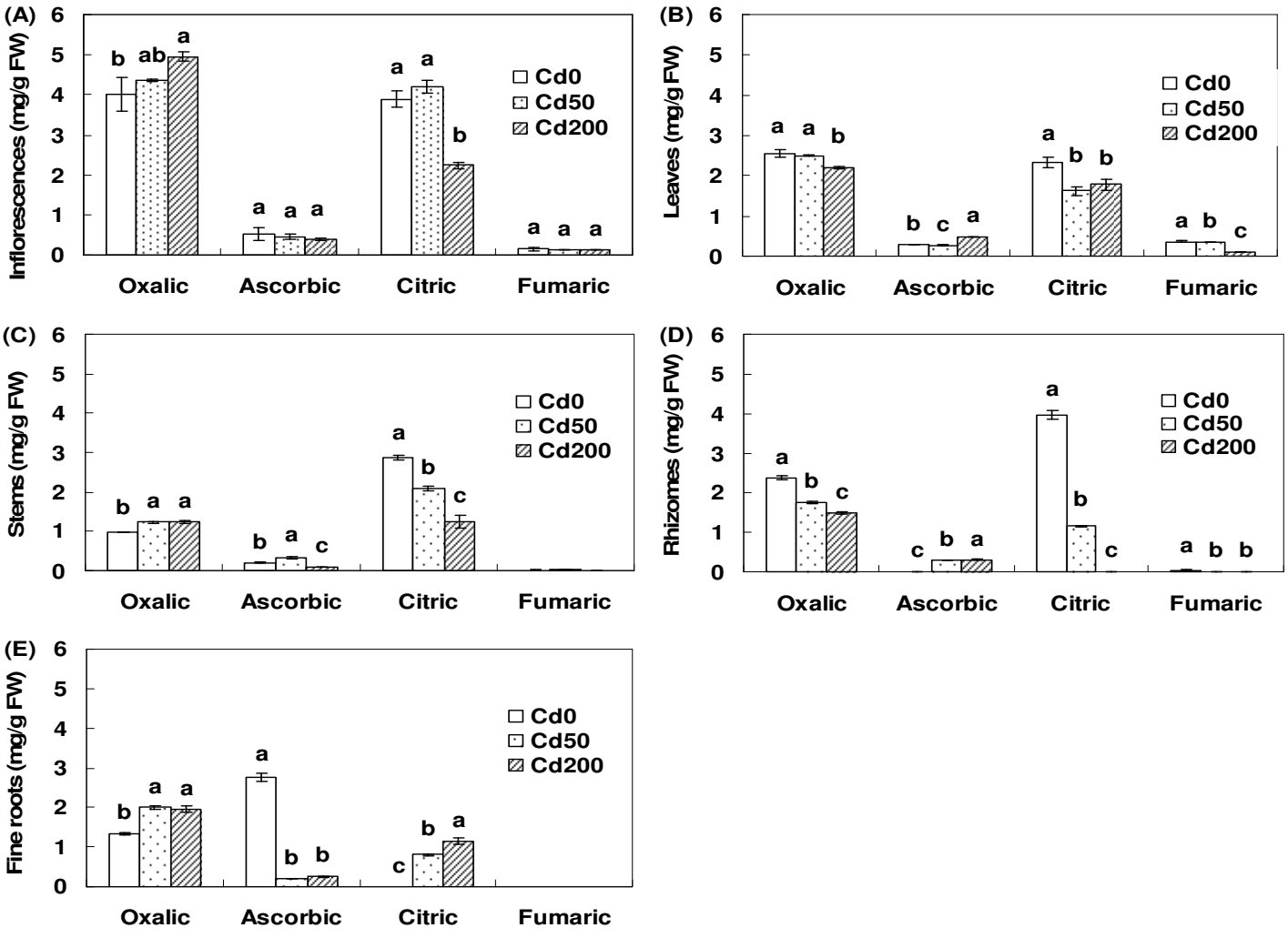

Figure 2. The contents of organic acids in inflorescences (A), leaves (B), stems (C), rhizomes (D) and fine roots (E) of *Spartina alterniflora* under different Cd treatments. Values at each tissue having a different small letter are significantly different from each other ($p<0.05$). Bars denote standard deviation. Cd0～Cd200: 0, 50, 200 mg/kg Cd^{2+} concentrations.

Table 3. Pearson correlation coefficients between organic acids levels and Cd bioaccumulations in inflorescences, leaves, stems, rhizomes and fine roots of *Spartina alterniflora.*

Parameter	Organic acid	Oxalic acid	Ascorbic acid	Citric acid	Fumaric acid	Total Cd	Water-soluble Cd
Inflorescences	Oxalic acid	1					
	Ascorbic acid	-0.726*	1				
	Citric acid	-0.724*	0.509	1			
	Fumaric acid	0.229	-0.223	0.152	1		
	Total Cd	0.705*	-0.563	-0.576	-0.457	1	
	Water-soluble Cd	0.702*	-0.537	-0.553	-0.427	0.968**	1
Leaves	Oxalic acid	1					
	Ascorbic acid	-0.924**	1				
	Citric acid	0.418	-0.189	1			
	Fumaric acid	0.959**	-0.988*	0.318	1		
	Total Cd	-0.725*	0.586	-0.834**	-0.683*	1	
	Water-soluble Cd	-0.788*	0.665	-0.805**	-0.757*	0.956**	1
Stems	Oxalic acid	1					
	Ascorbic acid	-0.038	1				
	Citric acid	-0.858**	0.504	1			
	Fumaric acid	-0.217	0.848**	0.666	1		
	Total Cd	0.967**	-0.260	-0.945**	-0.431	1	
	Water-soluble Cd	0.966**	-0.124	-0.906**	-0.368	0.975**	1
Rhizomes	Oxalic acid	1					
	Ascorbic acid	-0.973**	1				
	Citric acid	0.997**	-0.970**	1			
	Fumaric acid	0.976**	-0.998**	0.975**	1		
	Total Cd	-0.951**	0.869**	-0.958**	-0.874**		
	Water-soluble Cd	-0.974**	0.947**	-0.984**	-0.953**	0.962**	1
Fine roots	Oxalic acid	1					
	Ascorbic acid	-0.988**	1				
	Citric acid	0.954**	-0.957**	1			
	Fumaric acid	0	0	0	0		
	Total Cd	0.981**	-0.975**	0.991**	0	1	
	Water-soluble Cd	0.908**	-0.891**	0.978**	0	0.967**	1

The values marked by the * are significantly different at $P < 0.05$. The values marked by ** are significantly different at $P < 0.01$.

were positive correlations between total Cd and water-soluble Cd bioaccmulations in rhizomes (r = 0.962**) and fine roots (r = 0.967**).

DISCUSSION

Growth of *S. alterniflora*

Cadmium, non-essential for plant growth, was considered to be one of the most phytotoxic heavy metal contaminants from leakage from mines and refineries of metals, industrial sewage discharge and use of fertilizers (Mclaughlin et al., 1996; Shafi et al., 2009). Cd could not only induce growth reduction, necroses of leaf and root, but also affect mineral nutrition absorption, chlorophyll metabolism and water balance in plants (Zhang and Huang, 2000). In general, foliar Cd concentration of 5 to 30 µg/g dry weight is regarded to be phytotoxic for most plant species (Kabata-Pendias and Pendias, 1992). In the present study, it was evident that no toxicity symptom was detected in *S. alterniflora* under Cd stress. However, the decreased tiller and spike numbers in Cd treatment groups indicated that both sexual and asexual reproduction of *S. alterniflora* were all restrained by Cd stress to some extent. There was general agreement that Cd caused the excessive accumulation of ROS in plant cell, and impaired the cell integrity, increasing membrane

permeability (Heiss et al., 2003). As a general indicator of lipid peroxidation, MDA accumulated when plants were exposed to oxidative stress (Chaoui et al., 1997). Our result shows that ELR and proline increased significantly under Cd stress, similar to conclusions of Kong et al. (1999) who found that increasing Cd^{2+} concentration promoted ELR and MDA in extract solution of maize leaves, thus, affected the integrity of plasma membrane. Previous reports showed that some organic solutes in plant (such as proline and soluble sugar) acted as osmoprotectants in adaptation to various stresses (Schat et al., 1997). In the present study, under treatment Cd200, the increased contents of proline and soluble sugar indicated that severe Cd stress may stimulate generation of osmoprotectants in *S. alterniflora*. In addition, *S. alterniflora* was tolerant to Cd stress through accumulation of many antioxidant enzymes, including peroxidase (POD), superoxide dimutase (SOD) and catalase (CAT) Li et al. (2009, 2011). The strong Cd enduring property may be related to that the physiological mechanism of *S. alterniflora* stressed by heavy metal, at least partly, be consistent with its salt stress. To the best of our knowledge, as a halophyte naturally grown in environments with excess of salt, *S. alterniflora* was better adapted to salt stress at the seashore than other glycophytic plants commonly selected for phytoremediation research (Li et al., 2010; Manousaki and Kalogerakis, 2011b).

Cd bioaccumulation

Early studies showed that halophytes may be used as clean up tools for the remediation of heavy metals and salt impacted soils, enduring and accumulating undesirable heavy metals (Ghnaya et al., 2007; Manousaki and Kalogerakis, 2011a, b). As an alien weed in China, *S. alterniflora* possessed strong resistance to a wide range of adverse climatic and edaphic conditions, such as high temperature (Shi and Bao, 2007), saline and alkaline stresses (Hester et al., 2001; Li et al., 2010). Furthermore, *S.alterniflora* could absorb large amounts of nitrogen (N) and phosphorus (P) in water, and inhibit excess breeding of undesirable cyanobacteria (Shen et al., 2008). In addition, enzymatic activity in rhizosphere of *S. alterniflora* may affect heavy metal cycling, and take part in phytoremediation of heavy metals (Reboreda and Caçador, 2008). Carbonell et al. (1998) found that dimethylarsinic acid was transferred to the shoots, while inorganic arsenicals and monomethyl arsenic acid were mainly accumulated in the roots of *S. alterniflora*. Windham et al. (2003) compared the heavy metal accumulations between *S. alterniflora* and *Phragmites australis*, and found that *S. alterniflora* transported less amounts of copper (Cu) and zinc (Zn) to tissues aboveground and did not appear to have a tighter restriction on upward movement of mercury (Hg) and chromium (Cr) comparing to that of *P. australis*. Hempel et al. (2008) investigated the heavy metal accumulation of *S. alterniflora* in Bahía Blanca estuary salt marsh, and found that most of Cd and Mn were distributed in the aerated parts, indicating an allocation process from the roots up to the leaves. Therefore, the bioaccumulation and translocation of heavy metals in *S. alterniflora* is heavy metal specific. In the present study, under Cd stress, fine roots was not affected significantly, and propagated into an extensive network to spread entirely through the vermiculite which is favorable for phytoremediation. Furthermore, most of Cd was accumulated into fine roots, which may be related to that the root endodermis absorb and hinder the transportation of Cd from root to photosynthesis sites (Burke et al., 2000). Therefore, retention or immobilization of high amount of Cd in fine roots of *S. alterniflora* may be regarded as an important protection mechanism against the diffusion of Cd into shoots of *S. alterniflora*. In addition, Cd contents in aboveground parts of *S. alterniflora* were all lower than the critical judging standard (100 μg/g) of Cd hyperaccumulator (Table 2), indicating that *S. alterniflora* might not take part in phytoextraction in geographic cycle of Cd.

Organic acid and Cd accumulation

In general, organic acid was involved in heavy metal bioaccumulation and detoxification of hyperaccumulators, such as *Arabidopsis halleri* (Sarret et al., 2002), *S. nigrum* (Sun et al., 2006), and *R. globosa* (Sun et al., 2011). In the present study, it was evident that oxalic and citric acids were positively related with Cd bioaccumulations in fine roots. Furthermore, positive relationships between oxalic acid content and Cd bioaccumulation were also detected in stems and inflorescences. It could be hypothesized that oxalic acid may take part in chelation with Cd^{2+} and act as a carrier for Cd transport from underground to aboveground parts of *S. alterniflora*. Citric acid was negative to Cd accumulation in leaves of Cd-hyperaccumulator *S. melongena*, while was responsible for Cd accumulation in leaves of *S. nigrum* (Sun et al., 2006). In the present study, it is evident that citric acid content in fine roots increased with increasing Cd^{2+} concentration, indicating that citric acid may be an indicator of Cd bioaccumulation in fine roots. The positive correlations between ascorbic acid contents and Cd bioaccumulations revealed that ascorbic acid may also play an important role in Cd bioaccumulation. In addition, there was no significant positive correlation between organic acids and Cd bioaccumulations in leaves. Other strategies were possible, such as cell compartmentation and chelation with other metal ligands (nonprotein thiols, phytochelations, metallothionein and amino acids).

When stressed by heavy metals, detoxification of metal organic ligand complexes was one of the most effective strategies in heavy metal adapted plants (Sanita and

Table 4. The molar rates between organic acids and total Cd accumulations in different tissues of *Spartina alterniflora*

Cd treatments (mg/kg)	Inflorescences	Leaves	Stems	Rhizomes	Fine roots
0	20584.87	8576.62	6304.63	10445.21	3556.49
50	1716.60	2484.17	1431.02	1254.11	32.88
200	1162.80	1797.38	779.29	381.85	12.36

Gabbrielli, 1999). Generally speaking, metal ligands in plant included organic acids, amino acids, phytochelatins and metallothionein (Rauser, 1999). In the present study, organic acid / Cd molar rates were obviously higher than 1 in various tissues of *S. alterniflora* (Table 4), which indicated that these ligands were abundant enough to bind all Cd atoms in *S. alterniflora*. However, the organic acid / Cd molar rates in fine roots were lower compared with other tissues. In addition, the decreased trends of organic acid / Cd molar rates with increasing Cd^{2+} concentration were also detected. All that mentioned above may be resulted from that organic acids are not the only reason for Cd bioaccumulation and tolerance.

Previous studies showed that the water soluble Cd in leaves of *S. nigrum*, *R. globosa* and *R. islandica* was associated with organic acids (Sun et al., 2006, 2011). In the present study, with increasing Cd^{2+} concentration, proportion of Cd bioaccumulation that was water extractable in leaves and fine roots increased, suggesting that more and more Cd bioaccumulation is associated with water soluble compounds including organic acids. However, water-soluble Cd / total Cd decreased in stems and rhizomes, indicating that other metabolic and cellular strategies may also be existed in Cd detoxification. In addition, the stable water-soluble Cd / total Cd bioaccumulation in inflorescences and leaves showed the balance between various strategies under Cd stress.

In conclusion, *S. alterniflora* may be a promising candidate for phytoremediation of Cd-contaminated estuary wetlands. *S. alterniflora* could acclimate to Cd stress to some extent, and sequestrate most of Cd in underground parts. Organic acids (oxalic, citric and ascorbic acids) in *S. alterniflora* might play an important role in Cd tolerance and accumulation. However, further studies are still necessary to determine the accumulation forms of Cd and the cellular distribution in *S. alterniflora*.

ACKNOWLEDGMENT

This work was financially supported by the Program of National Natural Science Foundation of China (30470179) and Science Research Planning Project of Tianjin Port.

REFERENCES

Burke DJ, Weis JS, Weis P (2000). Release of metals by the leaves of the salt marsh grasses *Spartina alterniflora* and *Phragmites australis*. Estuar. Coast. Shelf. S. 51: 153-159.

Carbonell AA, Aarabi MA, DeLaune RD, Gambrell RP, Patrick Jr WH (1998). Arsenic in wetland vegetation: availability, phytotoxicity, uptake and effects on plant growth and nutrition. Sci. Total. Environ. 217: 189-199.

Cawthray GR (2003). An improved reversed-phase liquid chromatographic method for the analysis of low-molecular mass organic acids in plant root exudates. J. Chroomatogr. A, 1011: 233-240.

Chaoui A, Mazhoudi S, Ghorbal MH, Ferjani EEL (1997). Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus ulgaris* L.). Plant. Sci. 127:139-147.

Chung CH (2006). Forty years of ecological engineering with *Spartina* plantations in China. Ecol. Eng. 27: 49-57.

Costanza R, dArge R, deGroot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, Oneill VR, paruelo J, Raskin RG, Sutton P, Vanden Belt M (1997). The value of the world's ecosystem services and natural capital. Nature, 387: 253-260.

Cuong DT, Bayen S, Wurl O, Subramanian K, Wong KKS, Sivasothi N, Obbard JP (2005). Heavy metal contamination in mangrove habitats of Singapore. Mar. Pollut. Bull. 50: 1732-1738.

Dary M, Chamber-Pirez MA, Palomares AJ, Pajuelo E (2010). "In situ" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria. J. Hazard. Mater. 177: 323-330.

Defew LH, Mair JM, Guzman HM (2005). An assessment of metal contamination in mangrove sediments and leaves from Punta Mala Bay, Pacific Panama. Mar. Pollut. Bull. 50: 547-552.

Ghnaya T, Slama I, Messedi D, Grignon C, Ghorbel MH, Abdelly C (2007). Cd-induced growth reduction in the halophyte *Sesevium portulacastrum* is significantly improved by NaCl. J. Plant. Rres. 120: 309-316.

Hao ZB, Cang J, Xu Z (2004). Plant Physiology Experiment. Harbin Institute of Technology Press, Harbin.

Heiss S, Wachter A, Bogs J, Cobbett C, Rausch T (2003). Phytochelatin synthase (PCS) protein is induced in *Brassica juncea* leaves after prolonged Cd exposure. J. Exp. Bot. 54: 1833-1839.

Hempel M, Botté SE, Negrin VL, Chiarello MN, Marcovecchio JE (2008). The role of the smooth cordgrass *Spartina alterniflora* and associated sediments in the heavy metal biogeochemical cycle within Bahía Blanca estuary salt marshes. J. Soils Sediments, 8: 289-297.

Hester MW, Mendelssohn IA, McKee KL (2001). Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physical constraints. Environ. Exp. Bot. 46: 277-297.

Kabata-Pendias A, Pendias H (1992). Trace elements in soils and plants, 2nd ed. CRC Press, Boca Raton, Florida.

Kong XS, Zhang MX, Guo XP (1999). Effects of Cd toxicity on cell membrane permeability of maize seedlings and protective enzyme activities. Agro-environmental. Protection, 18:133-134.

Kramer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996). Free histidine as a metal chelator in plants that accumulate nickel. Nature, 379: 635-638.

Kramer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000). Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. Plant. Physiol. 122: 1343-1353.

Küpper H, Zhao FJ, McGrath SP (1999). Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant. Physiol. 119: 305-311.

Li JM, Shi GY, Wei Y (2011). Effects of cadmium on growth and physiological characteristics of *Spartina alterniflora*. J. Anhui. Agri. Sci. 39: 2174-2176, 2128.

Li LX, Zhao JQ, Chen SH, Pu YT, Guo SL (2009). Physiological response of *Spartina alterniflora* L. to heavy metal Cd stress. J Yantai Univ. Nat. Sci. Eng. 22: 328-332.
Li RL, Shi FC, Fukuda K (2010). Interactive effects of various salt and alkali stresses on growth, organic solutes, and cation accumulation in a halophyte *Spartina alterniflora* (Poaceae). Environ. Exp. Bot. 68: 66-74.
Liu AZ, Zhou DS, Liu F (2011). Cadmium tolerance and enrichment characteristics of *Eulaliopsis binata*. Chin. J. Appl. Ecol. 22: 473-480.
Lombi E, Zhao FJ, Dunham SJ, Mcgrath SP (2000). Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesinsense*. New. Phytol. 145: 11-20.
Lutts S, Kiner JM, Bouharmont J (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot. 78: 389-398.
Manousaki E, Kalogerakis N (2011a). Halophytes present new opportunities in phytoremediation of heavy metals and saline soils. Ind. Eng. Chem. Res. 50: 656-660.
Manousaki E, Kalogerakis N (2011b). Halophytes-an emerging trend in phytoremediation. Int. J. Phytoremediat. 13: 959-969.
Manousaki E, Kokkali F, Kalogerakis N (2009). Influence of salinity on lead and cadmium accumulation by the salt cedar (Tamarix smyrnensis Bunge). J. Chem. Technol. Biotechnol. 84: 877-883.
McLaughlin MJ, Tiller KG, Naidu R, Stevens DP (1996). Review: The behaviour and environmental impact of contaminants in fertilizers. Aust. J. Soil. Res. 34: 1-54.
Perronnet K, Schwartz C, Gérard E, Morel JL (2000). Availability of cadmium and zinc accumulated in the leaves of *Thlaspi caerulescens* incorporated into soil. Plant. Soil. 227: 257-263.
Rauser WE (1999). Structure and function of metal chelators produced by plants. Cell. Biochem. Biophys. 31: 19-48.
Reboreda R, Caçador I (2008). Enzymatic activity in the rhizosphere of *Spartina martima*: Potential contribution for phytoremediation of metals. Mar. Environ. Res. 65: 77-84.
Salla V, Hardaway CJ, Sneddon J (2011). Preliminary investigation of *Spartina alterniflora* for phytoextraction of selected heavy metals in soils from Southwest Louisiana. Microchem. J. 97:207-212.
Sanità di Toppi L, Gabbrielli R (1999). Response to cadmium in higher plants. Environ. Exp. Bot. 41: 105-130.
Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann JL, Traverse A, Marcus MA, Manceau A (2002). Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. Plant. Physiol. 130: 1815-1826.
Schat H, Sharma SS, Vooijs R (1997). Heavy metal-induced accumulation of free proline in a metal-tolerant and a nontolerant ecotype of *Silene vulgaris*. Physiol. Planta. 10: 477-482.
Schwitzguébel JP, Kumpiene J, Comino E, Vanek T (2009). From green to clean: a promising and sustainable approach towards environmental remediation and human health for the 21 st century. Agrochimica, 53: 209-237.
Shafi M, Bakht J, Hassan MJ, Raziuddin M, Zhang G (2009). Effect of cadmium and salinity stresses on growth and antioxidant enzyme activities of wheat (*Triticum aestivum* L.). Bull. Environ. Contam.Toxicol. 82: 772-776.
Shen YM, Yang JS, Zeng H, Zhou Q (2008). Review of studies on alien species *Spartina alterniflora* in China. Mar. Environ. Sci. 27: 391-396.
Shi FC, Bao F (2007). Effects of salt and temperature stress on ecophysiological characteristics of exotic cordgrass, *Spartina alterniflora*. Acta. Ecol. Sin. 27: 2733-2741.
Sun RL, Zhou QX, Jin CX (2006). Cadmium accumulation in relation to organic acids in leaves of *Solanum nigrum L.* as a newly found cadmium hyperaccumulator. Plant. Soil. 285: 125-134.
Sun RL, Zhou QX, Wei SH (2011). Cadmium accumulation in relation to organic acids and nonprotein thiols in leaves of recently found Cd hyperaccumulator *Rorippa globosa* and the Cd-accumulating plant *Rorippa islandica*. J. Plant. Growth, Regul. 30: 83-91.
Ueno D, Ma JF, Iwashita T, Zhao Fj, McGrath SP (2005). Identification of the form of Cd in the leaves of a superior Cd accumulating ecotype of *Thlaspi caerulescens* using Cd-NMR. Planta. 221: 928-936.
Wei SH, Jaime A, Teixeira da S, Zhou QX (2008). Agro-improving method of phytoextracting heavy metals contaminated soil. J. Hazard. Mater. 150: 662-668.
Weis JS, Weis P (2004). Metal uptake, transport and release by wetland plants: implications for phytoremediation and restoration. Environ. Int. 30: 685-700.
Windham L, Weis JS, Weis P (2003). Uptake and distribution of metals in two dominant salt marsh macrophytes, *Spartina alterniflora* (cordgrass) and *Phragmites australis*(common reed). Estuar. Coast. Shelf. S. 56: 63-72.
Yang XO, Feng Y, He ZL, Stoffella PJ (2005). Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J. Trace. Elem. Med. Bio. 18: 339-353.
Zhang JB, Huang WN (2000). Advances on physiological and ecological effects of cadmium on plants. Acta. Ecol. Sin. 20: 514-523.
Zhou QX, Song YF (2004). Principles and methods of contaminated soil remediation, Science press, Beijing.
Zhu DT, Li MH, Li X (2010). Cycle and enrichment characteristics of *Spartina alterniflora* community on heavy metal elements. J. Anhui. Agri. Sci. 38: 1203-1205, 1208.
Zhu YX, Wei YZ, Ye ZQ, Yang XE (2006). Function of organic acids in heavy metal tolerance mechanism in hyperaccumulator. J. Northwest Sci-Tech Univ. Agri. For. (Nat. Sci. Ed.) 34: 121-126.